ALKALOIDS OF PAPAVER SOMNIFERUM L.
BIOSYNTHETIC STUDIES AND CHARACTERIZATION OF NEW OPIUM ALKALOIDS

by

Cherng - Chyi Fu

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Pharmaceutical Chemistry

in the

GRADUATE DIVISION

(San Francisco)

of the

UNIVERSITY OF CALIFORNIA

Approved: Eric G. Brochmann-Hanssen

Chin-Fu Peng

Neal Castagnoli Jr.

Committee in Charge

Deposited in the Library, San Francisco Medical Center:

5-25-72 Josette S. Yeazell

Date Librarian

Degree Conferred: June 16, 1970

DISSERTATION ABSTRACT

ALKALOIDS OF PAPAVER SOMNIFERUM L.

Biosynthetic Studies and Characterization of New Opium Alkaloids

Cherng-Chyi Fu
Department of Pharmaceutical Chemistry
University of California, San Francisco

The objective of this study was to investigate the biosynthetic pathways and mechanisms of certain opium alkaloids and to explore the presence of new alkaloids in Papaver somniferum based on a biosynthetic approach.

Radioactive precursors (±)-reticuline-(N-$^{14}CH_3$), (±)-reticuline-(3-^{14}C), (±)-codamine-(3-^{14}C) and (±)-laudanine-(N-$^{14}CH_3$) were synthesized and fed to opium poppies. Reverse isotope dilution technique was applied to isolate the desired alkaloids. Isoboldine, coreximine, canadine and oxycryptopine were synthesized and used for isotope dilution purposes, the other alkaloids were obtained from commercial sources.

I. Biosynthetic studies

(A). Barton and Cohen proposed that aporphines are produced in plants from a benzyltetrahydroisoquinoline by a phenol

oxidation *via* an intermediate quinoid biradical. The possible mechanisms were envisaged, either a direct coupling or formation of an intermediate dienone which in turn might rearrange to give rise to a great variety of aporphines.

When (±)-reticuline-(3-^{14}C) was administed to opium poppies, it was incorporated into isoboldine to an extent of 0.073%. The feeding experiment was repeated with (±)-reticuline-(*N*-$^{14}CH_3$), isoboldine and magnoflorine being used for isotope dilution. Again, isoboldine showed good incorporation (0.084%) of radioactivity whereas magnoflorine was inactive. Since reticuline, isoboldine and magnoflorine are known to exist in the opium poppy, it may be concluded that aporphines with 1, 2, 9, 10 substitution pattern (isoboldine type) are biosynthesized by a direct phenol coupling, while this is not the case for aporphines with substituents at positions 1, 2, 10 and 11 (corytuberine type). Because of steric factors, these aporphines are more likely to be biosynthesized *via* dienone intermediate, followed by dienone-phenol rearrangement.

(B). By feeding opium poppies with (±)-codamine-(3-^{14}C) and (±)-laudanine-(*N*-$^{14}CH_3$), it was found that the major pathway leading from (+)-reticuline to (+)-laudanosine is by way of (+)-laudanine. The biosynthetic pathway leading from (+)-reticuline to (+)-laudanosine *via* (+)-codamine represents only a minor pathway.

II. Characterization of New Opium Alkaloids

(A). It has been shown that protoberberines are produced

in plants from (+)-reticuline in such a way that the N-methyl group of reticuline becomes the C-8 methylene group of the protoberberine. We have shown in this study that the tetrahydro-ψ-berberine, coreximine, is also derived from reticuline. (±)-Reticuline-(3-^{14}C) was incorporated into coreximine to an extent of 0.17%. Controlled degradation showed that the radioactivity was located at the C-6 position, as expected. Consequently, it could be concluded that the opium poppy is capable of converting reticuline to coreximine, and that coreximine, like scoulerine and isocorypalmine, is a normal member of the opium alkaloids. In the same way, it was shown that canadine, tetrahydropalmatine and berberine were not present in the plant.

(B). Our tracer studies showed no incorporation of (±)-reticuline-(N-$^{14}CH_3$) into glaucine, isocorydine, boldine, sinomenine and oxycryptopine. It would, therefore, seem logical to conclude that the opium poppy does not contain glaucine, boldine, sinomenine and oxycryptopine. No decision can be made, however, regarding the presence of isocorydine, since it, like corytuberine and magnoflorine, does not appear to be biosynthesized from reticuline in this plant.

TABLE OF CONTENTS

LIST OF SCHEMES

LIST OF TABLES

ACKNOWLEDGEMENT

It is a great pleasure to express my deep appreciation to Professor Einar Brochmann-Hanssen for his guidance, encouragement and his limitless help in the preparation of this thesis. I would like to acknowledge with sincere thanks the generous help and valuable suggestions of Professors Chin-Tzu Peng and Neal Castagnoli. I am also indebted to Dr. Lutfi Misconi, Dr. Albert Y. Leung and Dr. Galal Zanati for their assistance in the synthesis of some of the compounds used in this research project.

Last but not least, I wish to express my sincerest appreciation to my wife, Ching-mei, for doing a wonderful job in conducting my responsibilities on the domestic front. Her sense of sacrifice and cooperation has contributed greatly toward this project.

This work was supported by a research grant M. H. 03487, from the National Institute of Mental Health, U. S. Public Health Service Bethesda, Md.

Part I. INTRODUCTION TO THE OPIUM ALKALOIDS

Opium is the dried latex of the unripe capsules of Papaver somniferum L. The plant is an annual herb indigenous to Asia Minor. The opium alkaloids have been of interest to mankind since the beginning of recorded history. Its main constituent, morphine, has been referred to as "God's medicine", which serves to emphasize the important nature of this alkaloid especially for the relief of pain. Morphine is still used extensively in modern medicine. Because of the abundance and wide variety of alkaloids in opium, chemists and biochemists have long been engaged in intensive study of these alkaloids. More than one hundred and sixty years have passed since Sertürner (1) first isolated morphine in 1806. The other principal opium alkaloids, narcotine, codeine, thebaine and papaverine, were also discovered during the first half of the 19th century. So far, more than thirty-five opium alkaloids have been isolated, and the list continues to grow.

The opium alkaloids are classified into eight major groups.These are benzylisoquinolines (e.g., papaverine), hydrophenanthrenes (e.g., morphine), protopines (e.g., protopine), phthalideisoquinolines (e.g., narcotine), protoberberines (e.g., scoulerine), aporphines (e.g., corytuberine), benzophenanthridines (e.g., sanquinarine) and papaverrubines (e.g., porphyroxine). No member of the benzophenanthridines has actually been isolated as yet, but Hakim (2)

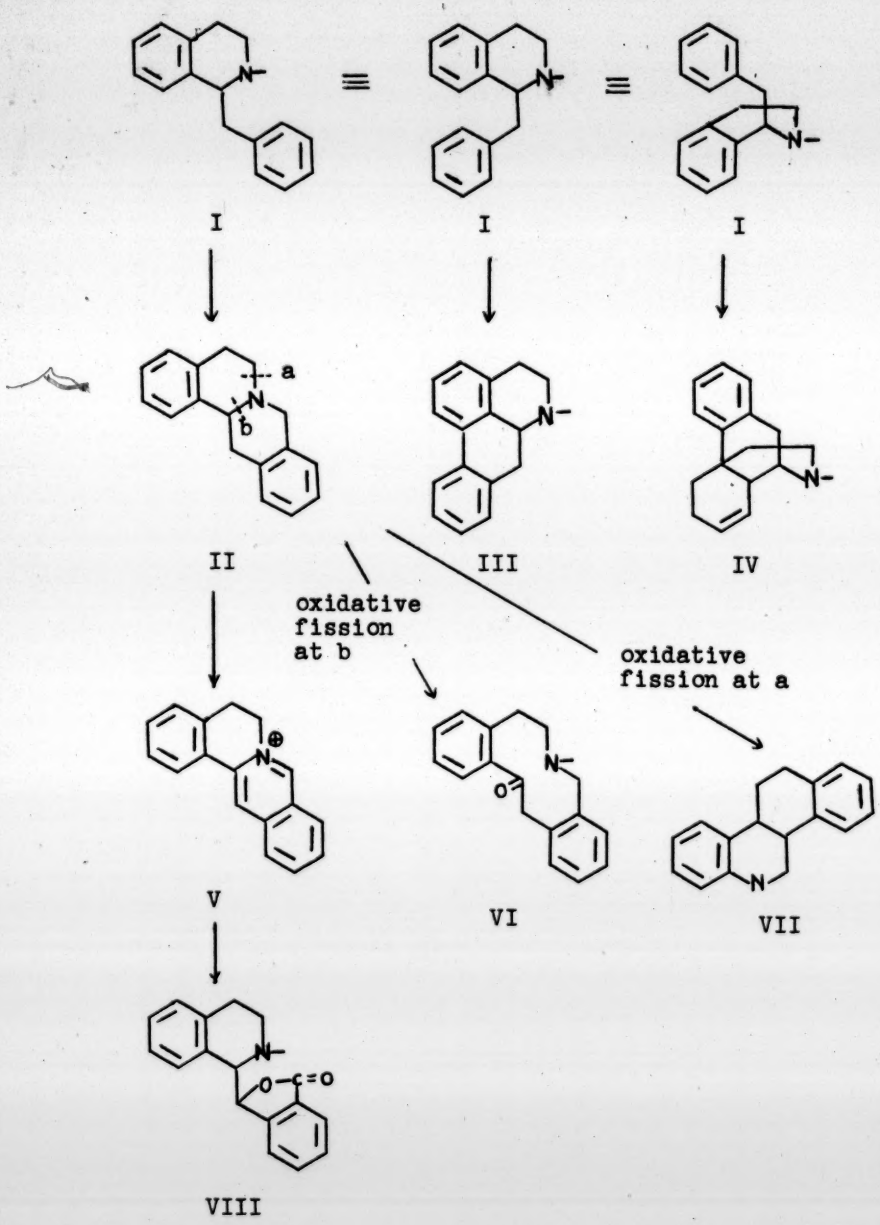
has claimed to have detected the presence of sanquinarine in opium by paper chromatography and paper electrophoresis.

During the past decades scientists have turned their attention to speculation of the ways in which these alkaloids are constructed in plants. It was Robinson who proposed the biogenetic theory through his structural correlations of many widely different alkaloids (3, 4). He recognized the structural relationships between the benzylisoquinolines (I), the hydrophenanthrenes (IV), and aporphines (III). He also perceived that the tetrahydroprotoberberine nucleus (II), was related to the skeletons of protopines (VI), phthalideisoquinolines (VIII), as well as to benzophenanthridines (VII). Scheme I illustrates these structural relationships.

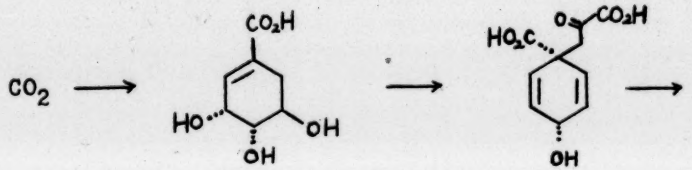
An idea which was going to have important consequences for later studies in alkaloid biosynthesis, was set forth by Winterstein and Trier (5) in 1910. They proposed that the benzylisoquinoline system in nature is built up from two units of 3,4-dihydroxyphenylalanine (DOPA) (XIV), which by decarboxylation and oxidative deamination were believed to give rise to 3,4-dihydroxyphenethylamine (dopamine) (XV) and 3,4-dihydroxyphenylacetaldehyde, respectively.

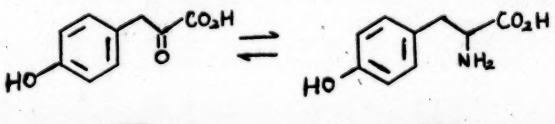
Modern theories (6) consider the reacting units to be dopamine (XV) and 3,4-dihydroxyphenylpyruvic acid (XIII) formed from shikimic acid (IX) *via* prephenic acid (X). The sequence of biosynthesis of opium alkaloids may, therefore, be illustrated as shown in Scheme II.

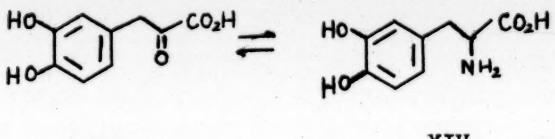
Scheme II indicates that norlaudanosoline (XVI) is the

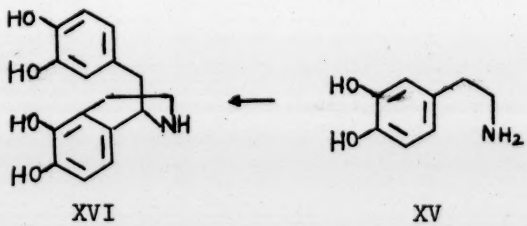

I
I
I
a
b
II
III
IV
oxidative fission at b
oxidative fission at a
V
VI
VII
VIII

Scheme I









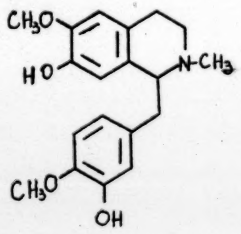
various groups of
opium alkaloids

Scheme II

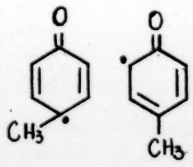
first "dimeric" intermediate in the biosynthetic sequence. The entry of this compound into alternative pathways leading to the various structural types of alkaloids is controlled by the presence or absence of different enzymes and the relative rates of these enzyme catalyzed processes. In addition, there appears to be a chemical control mechanism whereby norlaudanosoline is directed into competing routes leading to different structural variants. No data are available regarding the enzymology of these biosynthetic processes, but evidence is accumulating which can be interpreted in terms of chemical controls. Among them, the methylation step is one of the directing factors for chemical control which could limit the possibility of an ensuing oxidation reaction. A number of interesting experiments have been carried out by the schools of Barton and Battersby. Several methylated derivatives of norlaudanosoline have been prepared with labeled atoms and their incorporation into various alkaloids has been tested. Reticuline (XVII) was shown to give good incorporation into berberine (7), protopine (8) and thebaine (9), etc. Experiments with four isomeric O,O,N-trimethylnorlaudanosoline indicated that only reticuline could serve as a precursor of the hydrophenanthrene alkaloids (10). Furthermore, the isolation of reticuline (XVII) from opium in 1964 by Brochmann-Hanssen and Furuya (11), proved that reticuline is a normal constituent of the opium poppy. These results clearly indicated that reticuline has the proper methylation pattern to occupy a central position in the network of biosynthetic pathways.

Two types of reaction mechanisms have been used to explain the transformation of reticuline into various groups of alkaloids in opium. These are oxidative phenol coupling and Mannich type reactions.

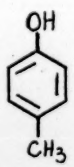
Barton and Cohen (12) suggested that Pummerer's ketone (XVIII) is formed by the coupling of phenoxy radicals (XIX) obtained in the ferricyanide oxidation of p-cresol (XX).



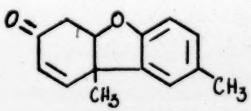
XVII



XIX

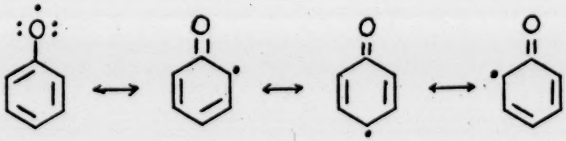


XX



XVIII

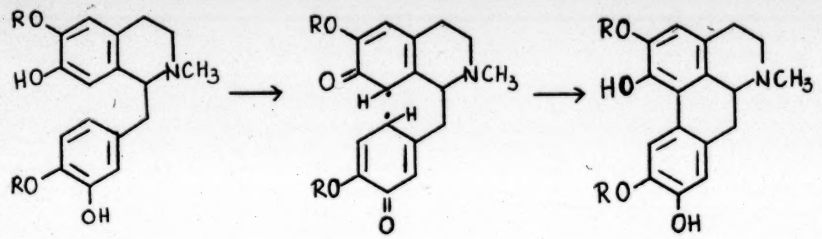
The oxidation of phenols by a one-electron transfer generates phenolic radicals which are comparatively stable due to the delocalization of the odd electron over the ortho and para positions of the aromatic nucleus (XXI). The coupling of two such radicals provides a stable molecule.



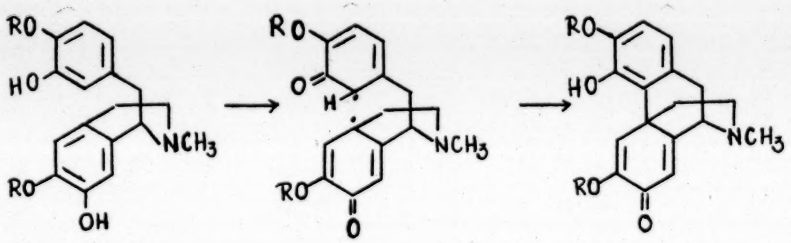
XXI

This concept has proved of utmost importance in predicting the course of biosynthetic pathways for a wide range of natural products. If one accepts the theory of radical pairing and also takes cognizance of the true structure of

Pummerer's ketone, one may predict the biogenetic pathways from benzylisoquinoline (XXII) to aporphine (XXIII) and to morphinane (XXIV) to be as follows:

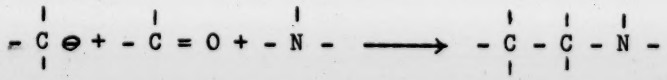


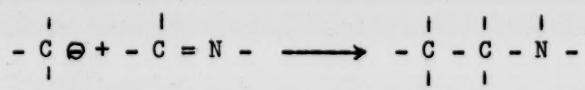
XXII ||| XXIII



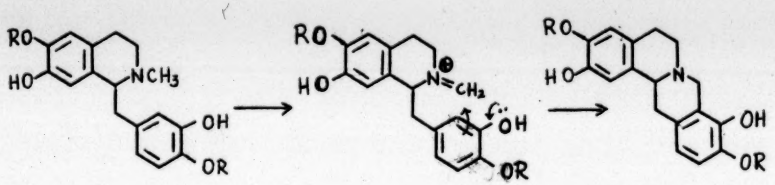
XXII XXIV

Robinson (3, 13) recognized that if a Mannich type reaction could occur in plants, it might explain a wide variety of alkaloid structures.





The biosynthesis of protoberberine alkaloids may be explained on this basis (XXII → XXV → XXVI).



XXII XXV XXVI

Because of the general availability of radioisotopes, it is possible to put the biogenetic hypotheses to test. The tracer technique has become a powerful tool for the study of the mechanisms and pathways by which natural products are produced. Most frequently the method consists of feeding plants with radioactive precursors and isolating the desired product. The radioactivity is determined as well as the position of the label in the molecule. Frequently, a reverse isotope dilution technique is applied to the biosynthetic study when the desired product appears in the plants in only a small amount. A known amount of alkaloid is added to the plant mash during extraction and is recovered by isolation, separation and purification.

The main aim of the study reported in this dissertation was to investigate the unknown pathways involved in the biosyntheses of certain opium alkaloids. Two possible pathways for the biosynthesis of laudanosine were investigated along with the mechanisms and pathways for the biosynthesis of aporphines. The biosynthetic relationship between reticuline

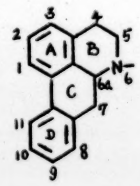
and oxycryptopine was also studied.

Another aspect of this investigation was to explore the presence of new alkaloids in the opium poppy. The detection of such minor alkaloids was based on a biosynthetic approach. The alkaloids under investigation were coreximine, canadine, berberine, tetrahydropalmatine, glaucine, isocorydine, boldine and sinomenine. Although these alkaloids have not yet been isolated from opium or the opium poppy, their presence is theoretically possible because of their biogenetic relationships to other known opium alkaloids. In order to investigate this possibility, specifically labeled reticuline was fed to the opium poppy, and non-radioactive alkaloids were added to the plant mash during the extraction. If the isolated alkaloids were radioactive and possessed the label in the predicted position, one might reasonably conclude that they were derived from reticuline and were normal constituents of the plant.

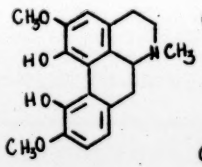
Part II. APORPHINE ALKALOIDS

A. Occurrence and Synthesis

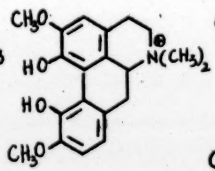
The structure of the aporphine alkaloids is based on the 4H-dibenzo (d,e,g) quinoline structure (XXVII). The numbering system which is in conformity with the "Ring Index", is as indicated.



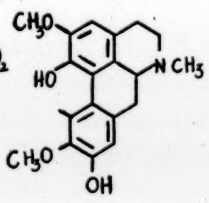
XXVII



XXVIII



XXIX

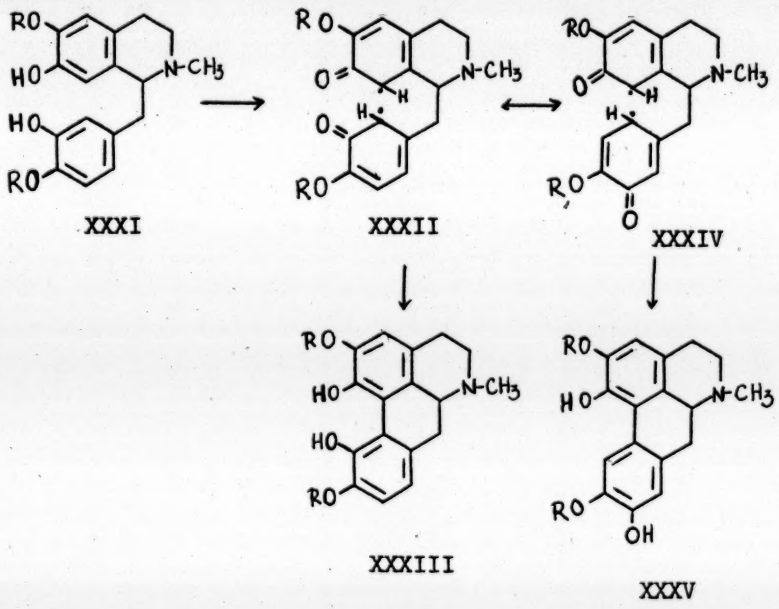


XXX

The aporphines occur most abundantly in the Papaveraceae, but they are also widely distributed in the Anonaceae, Lauraceae and Monimiaceae. Several aporphines are present in the opium poppy as minor alkaloids. In 1964, Nijland (14) isolated corytuberine (XXVIII) and magnoflorine (XXIX) from opium. Another aporphine alkaloid, isoboldine (XXX) was isolated by Brochmann-Hanssen et al (15) in 1967. On the basis of biosynthetic reasoning, one might expect more aporphine alkaloids to be present in the opium poppy.

Robinson (3) recognized the structural similarity between aporphines and benzylisoquinolines and suggested that the aporphine alkaloids are biogenetically derived from the

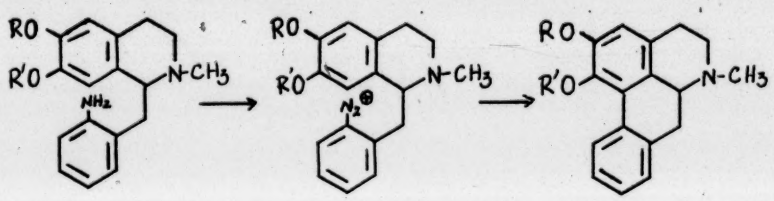
benzyltetrahydroisoquinolines. Barton and Cohen (12) proposed oxidative phenol coupling to be the mechanism for bond formation between ring A and ring D as illustrated in Scheme III.



Scheme III

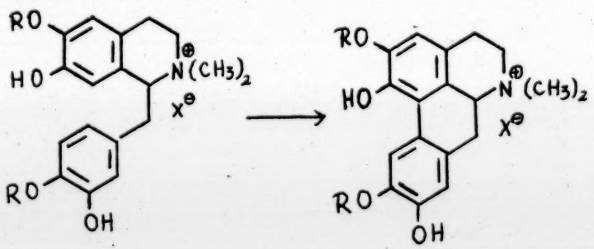
The chemical syntheses of aporphines normally follow Pschorr's procedure (16). The ring closure proceeds via diazotization of the amine (XXXVI) and nucleophilic reaction of the 8-position of the isoquinoline ring with the aromatic cation which is derived from the diazonium salt (XXXVII).

This method was further developed by Hey et al.(17) who synthesized a variety of phenolic aporphines by protecting the phenol group as the benzyl ether.



XXXVI XXXVII

Franck et al (18) reported the first biogenetic synthesis of an aporphine alkaloid based on Barton and Cohen's prediction regarding the intramolecular phenol coupling. They showed that oxidation of laudanosoline methiodide (XXXVIII, R=H) with aqueous ferric chloride solution gave compound (XXXIX, R=H) in 62% yield.



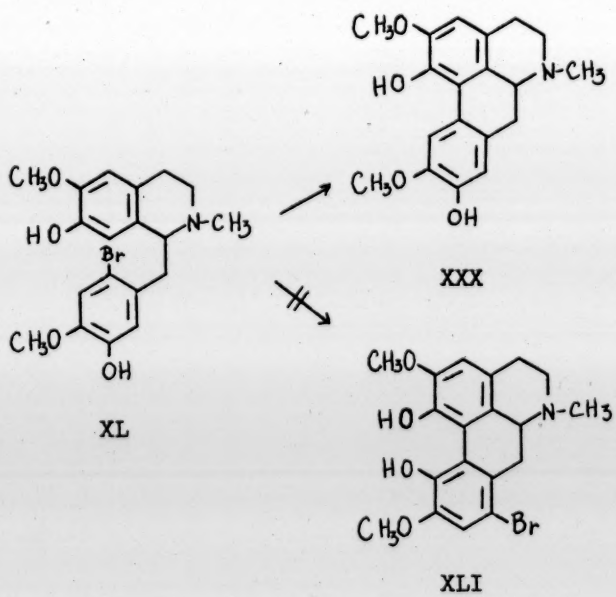
XXXVIII XXXIX

Similarly, Albonico et al (19) reported that oxidation of tembetarine chloride (XXXVIII, R=CH_3) gave laurifoline chloride (XXXIX, R=CH_3), although their yield was only 3%. Still lower yields were obtained if the nitrogen was tertiary. Thus, oxidation of reticuline (XVII) with potassium ferricyanide produced 0.5% of isoboldine (XXX) (18). This suggests that coupling is more successful in quaternary salts

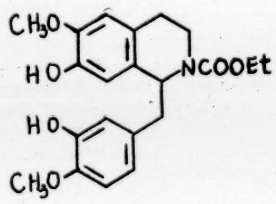
of benzylisoquinolines than in the tertiary bases by preventing carbon-nitrogen interactions and making carbon-carbon interactions more favorable.

So far, oxidative coupling of phenolic benzyltetrahydroisoquinolines has only produced aporphines substituted in positions 1,2,9 and 10. No aporphine alkaloid substituted at positions 1, 2, 10 and 11 has been obtained in this manner.

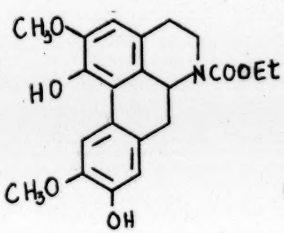
Jackson and Martin (20), investigated the oxidation of bromoreticuline (XL) in the expectation that the bromine would inhibit coupling <u>para</u> to the hydroxyl group and, therefore, favor <u>ortho</u> coupling with the formation of bromocorytuberine (XLI). However, the only product obtained was isoboldine (XXX) in 2.5% yield.



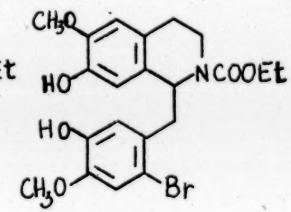
Recently, a very active research program on synthesis by oxidative phenol coupling has been carried out by Kametani and co-workers (21). The oxidative coupling of N-ethoxycarbonylnorreticuline (XLII) in a two-phase system of chloroform and aqueous potassium ferricyanide in ammonia gave 5-7% yield of aporphine (XLIII). The same product was obtained when bromo,N-ethoxycarbonylnorreticuline (XLIV) was oxidized under similar conditions. No trace of a 1,2,10, 11-substituted aporphine could be detected in either case.



XLII



XLIII



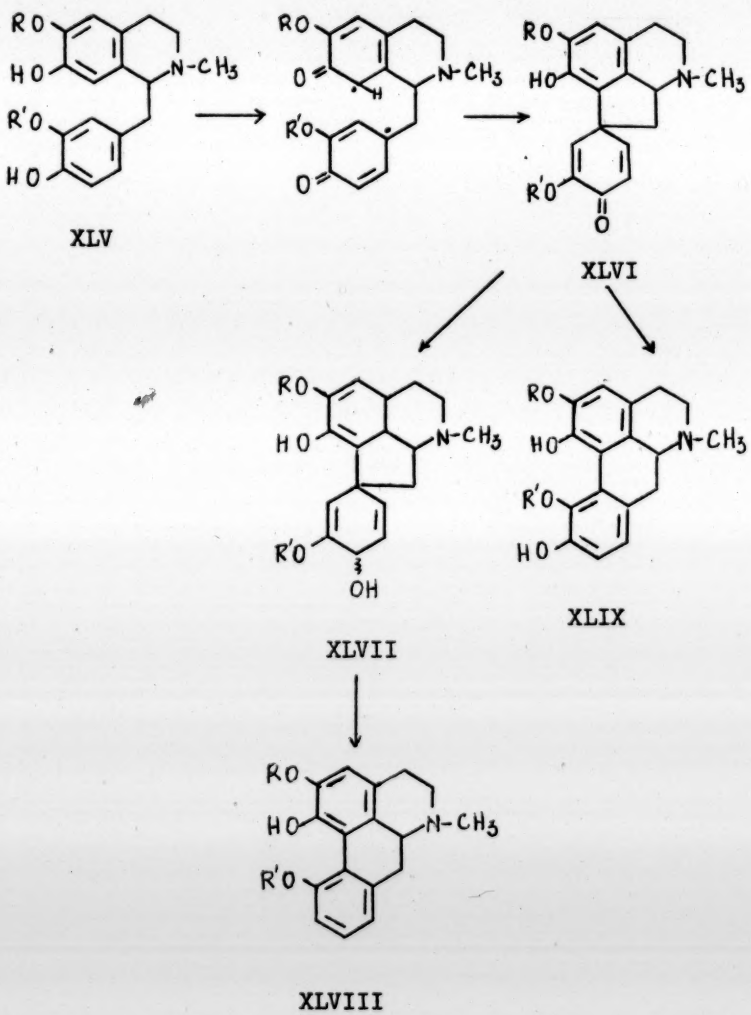
XLIV

Barton and Cohen (12) suggested that dienone (XLVI) might be an intermediate in the biosynthesis of some aporphines from benzylisoquinolines since such dienones might rearrange to the aporphine nucleus either through a dienone-phenol (XLVI — XLIX) or through a dienol-benzene rearrangement (XLVII — XLVIII) as illustrate in Scheme IV.

Experimental evidence for the dienol-benzene and dienone-phenol rearrangements *in vitro* has been furnished by Plieninger (22) and Auwers (23) respectively.

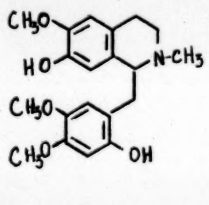
These ideas have been used to advantage for the synthesis of aporphines. Jackson and Martin (24) oxidized compound (L) with potassium ferricyanide and obtained the dienone (LI).

Scheme IV

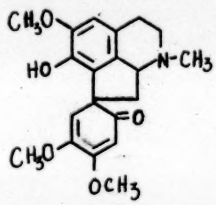


XLV XLVI XLVII XLIX XLVIII

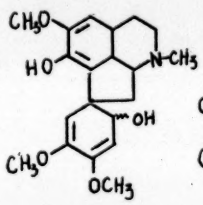
Reduction with sodium borohydride gave the dienol (LII) which underwent dienol-benzene rearrangement to form corydine (LIII). Shamma *et al* (25) methylated the dienone (LI) with diazomethane to the tetramethoxy base (LIV)..Reduction followed by rearrangement gave glaucine (LVI).



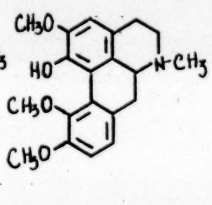
L



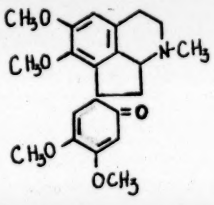
LI



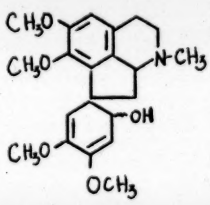
LII



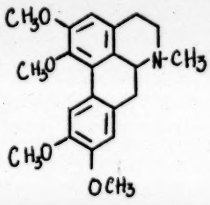
LIII



LIV



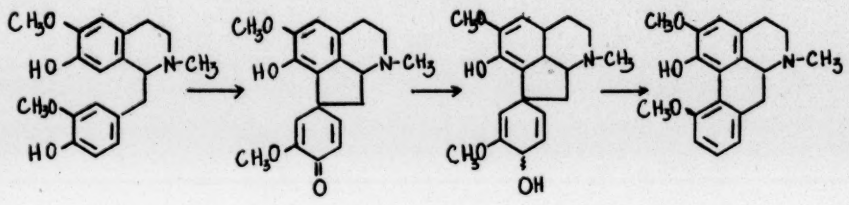
LV



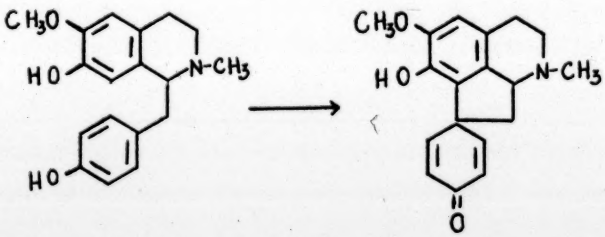
LVI

In a similar reaction sequence Battersby *et al* (26, 27) reported the synthesis of isothebaine (LX). Orientaline (LVII) was oxidized by alkaline ferricyanide to a mixture of two dienones (LVIII) in 4% yield. One of the dienones was reduced to dienol which rearranged by treatment with acid to give isothebaine (LX).

Kametani and Yagi (28) have reported the synthesis of the dienone, (±)-glaziovine (LXII) by oxidative coupling of (±)-*N*-methylcoclaurine (LXI) with potassium ferricyanide.



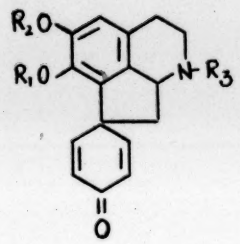
LVII LVIII LIX LX



LXI LXII

In vitro experiments gave strong support to the hypothesis that dienones are important intermediates in the formation of aporphines. Their biogenetic role was further appreciated by the discovery of dienone alkaloids in plants. The first such alkaloid, crotonosine (LXIII), was isolated in 1963 by Haynes and Stuart (29). Further alkaloids of this type are pronuciferine (LXIV) fugapavine (LXV), N-methylcrotonosine (LXVI), glaziovine (LXVII) and stepharine (LXVIII).

These are generally referred to as proaphorphines in view of their possible role as intermediates in the biosynthesis of aporphines.



LXIII: R_1=CH_3; R_2=R_3=H

LXIV: R_1=R_2=R_3=CH_3

LXV: R_1+R_2=-CH_2-; R_3=CH_3

LXVI: R_1=R_3=CH_3; R_2=H

LXVII: R_1=H; R_2=R_3=-CH_3

LXVIII: R_1= R_2=CH_3; R_3=H

B. Stereochemical Considerations

The apparent inability of the phenolic benzyltetrahydroisoquinolines to yield aporphines with substituents at positions 1, 2, 10 and 11 is probably due to the influence of steric factors which prevent direct coupling of radicals ortho to the two phenolic groups. Molecular models, UV, ORD and NMR spectroscopic evidence indicate that substituents at the 1 and 11 positions in aporphines cause considerable twisting of the biphenyl ring system from coplanarity. Bick et al (30) demonstrated that the methoxy groups of aporphines at positions 1 and 11 exhibit higher chemical shifts (3.4-3.72 ppm) than those at positions 2, 9 or 10 (3.72-3.89 ppm). This is also true for the benzylisoquinoline system. The higher chemical shifts of methoxy protons at positions 1 and 11 have been attributed to the steric factor responsible for the non-planarity of the biphenyl ring system, which in turn causes smaller deshielding by the aromatic ring current. Shamma (31, 32) reported that aporphines with a C-11 substituent manifest themselves by a

high specific rotation (at the sodium D line) and an UV absorption maximum at 270 mμ, while C-11 unsubstituted members show lower specific rotation as well as a bathochromic shift. This reflects the rigid and strained biphenyl system existing in the 1,2,10,11-substituted aporphines. Djerassi, Mislow and Shamma (32) have further reported that the twisted biphenyl chromophore of aporphines not only contributes to optical activity, but also plays an important role in Cotton effects that may be observed in the rotatory dispersion curves of aporphines. Thus, 1,2,9,10-tetrasubstituted aporphines belonging to the S-series show a negative Cotton effect at 320 mμ., whereas aporphines of the S-series having substituents at positions 1, 2, 10 and 11 show a positive Cotton effect. Based on these finding the absolute configuration of aporphines (magnoflorine, corytuberine and isoboldine) occurring in opium poppy, have been assigned to S-series (15, 32).

C. Biosynthetic Theories

Based on the evidence from <u>in vitro</u> biogenetic type syntheses, physical spectroscopic data and the isolation of "proaporphines" from plant materials, one may conclude that the biosynthesis of aporphine alkaloids may involve different processes depending on their substitution patterns.

Aporphines which are substituted at positions 1, 2, 9 and 10 (e.g., isoboldine, glaucine, etc.) may be biosynthesized by a direct <u>ortho-para</u> coupling of suitable

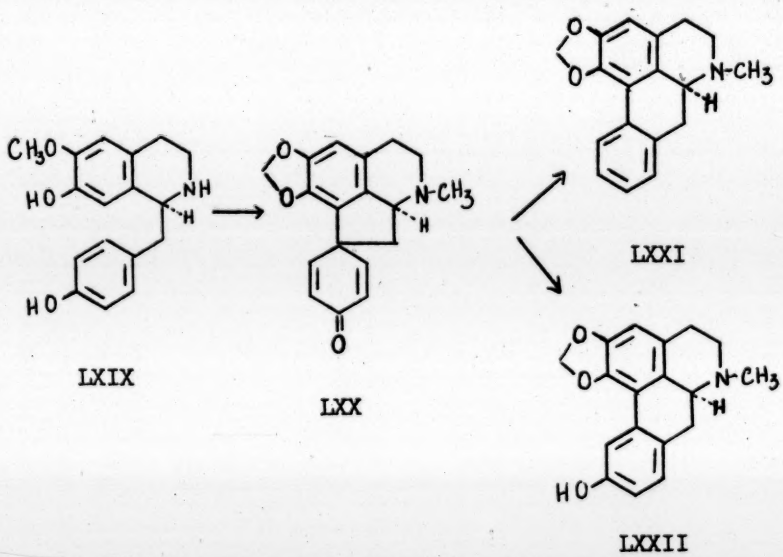
benzyltetrahydroisoquinolines as illustrated in Scheme III (page 11) (XXXI → XXXIV → XXXV).

On the other hand, the biosynthesis of aporphines having a 1,2,10,11-substitution pattern (e.g., corytuberine, magnoflorine, etc.) may proceed by way of a dienone intermediate followed by dienone-phenol rearrangement as illustrated in Scheme IV (page 15) (XLV → XLVI → XLIX).

Finally, alkaloids with no or only one substituent in ring D (e.g., isothebaine (LX)) may be derived from a suitable benzyltetrahydroisoquinoline by oxidation to a dienone, reduction to a dienol and rearrangement as illustrated in Scheme IV (page 15) (XLV → XLVI → XLVII → XLVIII).

Some of these proposed mechanisms have been subjected to in vivo studies by appropriate tracer experiments. Battersby et al. (33, 34) have shown that both orientaline (LVII) and orientalinone (LVIII) are efficiently incorporated into isothebaine (LX) in Papaver orientale. Administration of doubly labeled orientaline-(3-^{14}C; 3'-O-^{14}CH$_3$) gave isothebaine with the expected labeling pattern and without any change in the ratio of the two labels. Furthermore, (+)-orientaline which has the same absolute configuration as natural (+)-isothebaine, was incorporated twenty-eight times more efficiently than (-)-orientaline. Barton and his co-workers (35, 36) studied the incorporation of coclaurine (LXIX) and its N-methyl derivative into the corresponding proaporphines (dienones) and aporphines. When coclaurine-(8,3',5'-^{3}H$_3$) (LXIX) was administered to Papaver dubium, the radioactivity was incorporated into mecambrine (LXX)

and roemerine (LXXI). Labeled mecambrine was also incorporated into roemerine in Papaver dubium and into mecambroline (LXXII) in Meconopsis cambrica. This evidence confirms the biosynthetic route shown in the following scheme:



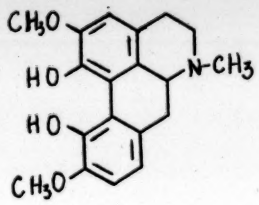
LXIX LXX LXXI LXXII

It may be concluded from the work of Battersby and Barton that dienones are involved in the biosynthetic pathways of certain aporphines via dienol-benzene and dienone-phenol rearrangements, namely those which are either unsubstituted or monosubstituted in ring D.

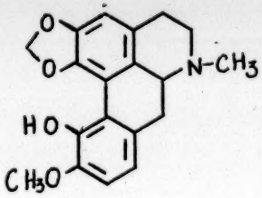
In contrast to this, Blaschke (37) reported a good incorporation (1.4%) of (±)-reticuline-(N-$^{14}CH_3$) into bulbocapnine (LXXIII) in Corydalis cava. This would seem to involve direct ortho-ortho coupling via corytuberine (XXVIII).

The biosynthesis of aporphine alkaloids is, therefore,

by no means fully understood.



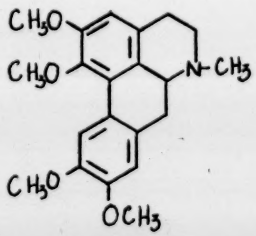
XXVIII



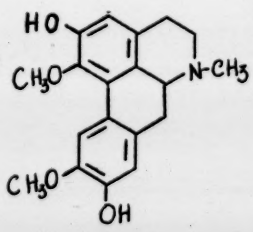
LXXIII

D. Potential Aporphine Alkaloids in Opium Poppy

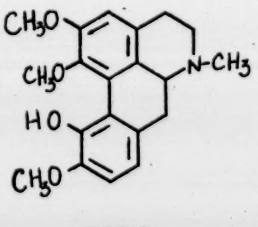
Several aporphine alkaloids, e.g., glaucine (LXXIV), boldine (LXXV) and isocorydine (LXXVI) which occur widely in nature, have never been found in opium. However, their close biogenetic relationships to isoboldine, corytuberine and magnoflorine would suggest that they might be present—although in very low concentration.



LXXIV



LXXV



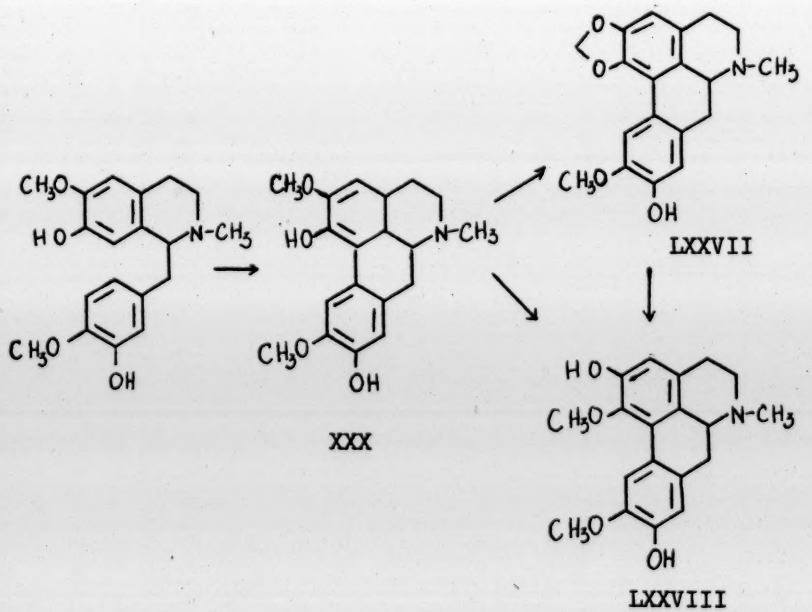
LXXVI

Glaucine is presumably biosynthesized from isoboldine by methylation of the phenolic groups. Incorporation of labeled reticuline into isoboldine and glaucine in the opium poppy would possibly mean that the direct ortho-para coupling

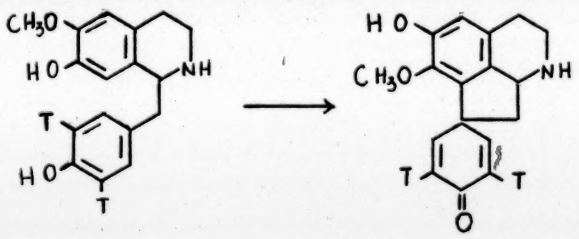
mechanism is operating in the plant; however, it would also show that glaucine is a normal alkaloid constituent of the plant.

The biosynthesis of isocorydine is related to corytuberine and magnoflorine. Therefore, the incorporation of labeled reticuline into isocorydine and magnoflorine would indicate that direct ortho-ortho coupling may take place in the plant and that isocorydine is a natural opium alkaloid.

Boldine is isomeric with isoboldine, however, the substitution pattern in ring A is reversed. It may be derived from isoboldine by one of two possible pathways, either via the methylenedioxy intermediate (LXXVII) or by demethylation and remethylation of ring A (XXX → LXXVIII).

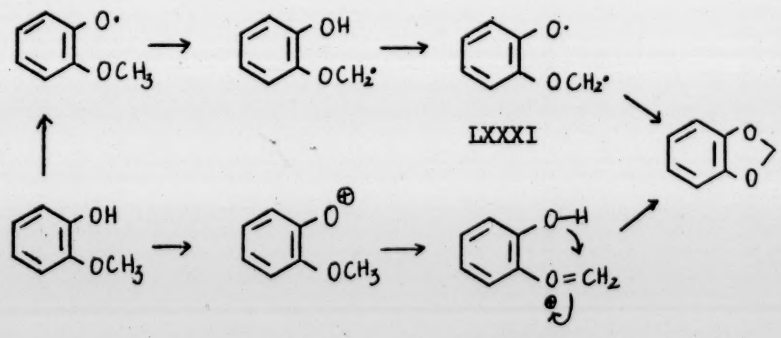


Alkaloids with the substitution pattern similar to (XXX, LXXVII, LXXVIII) frequently occur in the same plant or plant families. It has been shown by Barton et al. (38) that coclaurine (LXXIX) labeled with tritium was incorporated into the corresponding dienone, crotonosine (LXXX), in which the substitution pattern of ring A is reversed.



LXXIX LXXX

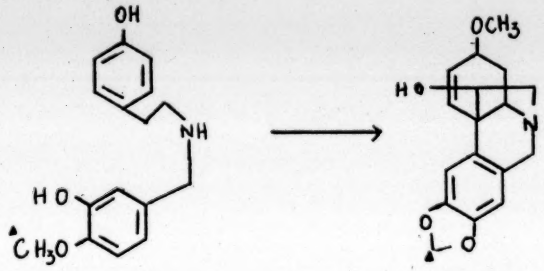
Sribney and Kirkwood (39) proposed that the methylenedioxy group may be produced in nature by a cyclization of ortho-methoxyphenol. Mechanistically, this may proceed by oxidation to the radical (LXXXI) or the cation (LXXXII) as shown below:



LXXXI LXXXII

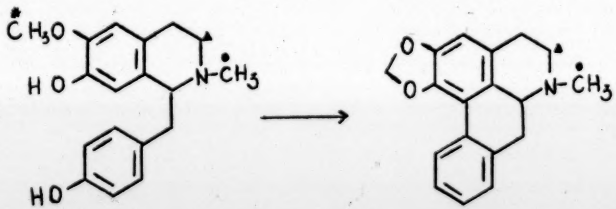
Barton et al. (40) reported the administration of (+)-norbelladine-(O-$^{14}CH_3$) (LXXXIII) in King Alfred daffodil

to yield ha manthamine (LXXXIV) labeled specifically in the methylenedioxy group.



LXXXIII LXXXIV

On the other hand, it has also been shown by Barton *et al.* (35) that N-methylcoclaurine-(N,O-methyl-3-^{14}C) fed to *Papaver dubium*, lost almost all radioactivity in the methylenedioxy group when incorporated into roemerine (LXXI).



LXXI

This was explained by assuming a rapid O-demethylation followed by remethylation from the unlabeled methylation pool of the plant and subsequent oxidative cyclization to the methylenedioxy ring.

E. Experimental Approach

Since the opium poppy contains reticuline as well as

diphenolic tetrasubstituted aporphines having both a 1, 2, 10,11-substitution pattern (corytuberine and magnoflorine) and a 1,2,9,10-substitution pattern (isoboldine) it would seem to lend itself to further study of aporphine biosynthesis. The purpose of our study was to investigate the possibility of a direct coupling in the biosynthesis of 1, 2,9,10- and 1,2,10,11-substituted aporphines in the opium poppy.

As an adjunct to our mechanistic investigation of the biosynthesis of aporphines, studies were carried out to detect the presence of the above mentioned potential aporphine alkaloids.

In order to achieve our objectives, the logical precursor is reticuline. Reticuline-(3-^{14}C) and reticuline-(N-$^{14}CH_3$) were used for feeding experiments. Plants were fed with labeled reticuline, and the alkaloids isoboldine, magnoflorine, boldine, isocorydine and glaucine were used for reverse isotope dilution during extraction, and subsequently isolated and purified. The radioactivity of the purified alkaloids and the location of the label were determined.

1. Synthesis of Labeled Precursors

(a) Reticuline-(N-$^{14}CH_3$).-- (41)

The starting materials were isovanillin (LXXXV) and vanillin (XCII). Benzylation with benzyl chloride under mildly basic condition yielded the corresponding O-benzyl

ethers (LXXXVI) (XCIII). The aldehyde group of the O-benzylisovanillin was reduced with sodium borohydride to the corresponding alcohol (LXXXVII) which was converted to the chloride (LXXXVIII) with thionyl chloride and reacted with potassium cyanide to give corresponding nitrile (LXXXIX). Hydrolysis with potassium hydroxide produced the acid (XC), which was converted to the acid chloride (XCI) with thionyl chloride.

O-benzylvanillin (XCIII) was reacted with nitromethane to give the nitrostyrene (XCIV) which was reduced with lithium aluminum hydride to the phenylethylamine (XCV). Reaction of this amine (XCV) with the acid chloride (XCI) under Schotten-Baumann condition yielded the amide (XCVI) which was cyclized with phosphorus oxychloride to the 1-benzyl-3,4-dihydroisoquinoline hydrochloride (XCVII). Liberation of the free base and reaction with iodomethane-(^{14}C) gave the methiodide (XCVIII) which was reduced with sodium borohydride to O,O-dibenzyltetrahydrobenzylisoquinoline (XCIX). The protecting benzyl group was removed by acid hydrolysis. The base was liberated by addition of ammonia and extraction with ether. The synthetic sequence is illustrated in Scheme V.

(b) (±)-Reticuline-(3-^{14}C).--

This compound was synthesized by Dr. G. Zanati, the synthesis scheme is illustrated in Scheme VI.

2. Aporphines for Reverse Isotope Dilution

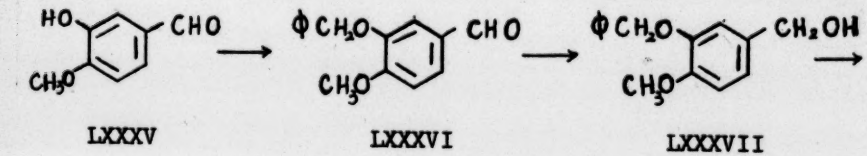
Magnoflorine was kindly supplied by Dr. Jack Beal. Glaucine, isocorydine and boldine were purchased from Pierce Chemical Company.

Isoboldine was synthesized by Dr. Lutfi Misconi by the modification of Pschorr ring closure reaction. The scheme of the synthesis is outlined in Scheme VII.

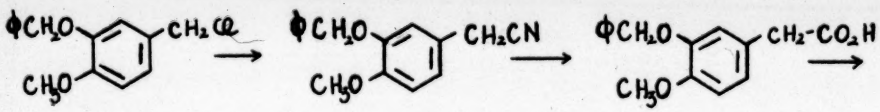
3. Feeding Procedure

The plants used for the studies were Papaver somniferum L. (Noordster and Indra varieties). These were grown in flower pots in a greenhouse and the feeding of the radioactive precursors was made at the end of the flowering season as soon as the petals had fallen. An aqueous solution of reticuline sulfate containing the equivalent of about 3 mg./ml. of reticuline base was injected into the top of the seed capsule as described by Battersby, Binks and Harper (42). In order to avoid swamping the metabolic pools and thus possibly cause abnormal biosynthetic behavior, each capsule received only 0.3 ml. of injection solution. The plants were allowed to grow normally for a further period of 1 to 2 weeks before they were harvested and stored in a deep freezer until such time as they were required for extraction.

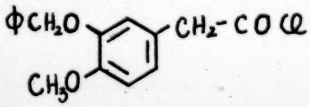
Three batches of plants were used. In the first batch, (±)-reticuline-(3-^{14}C) was fed to plants and the aporphine alkaloid (±)-isoboldine together with the protoberberines (±)-canadine and (±)-coreximine were added as diluents. In the



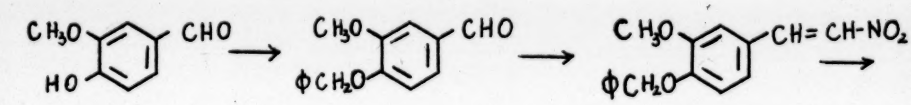
LXXXV LXXXVI LXXXVII



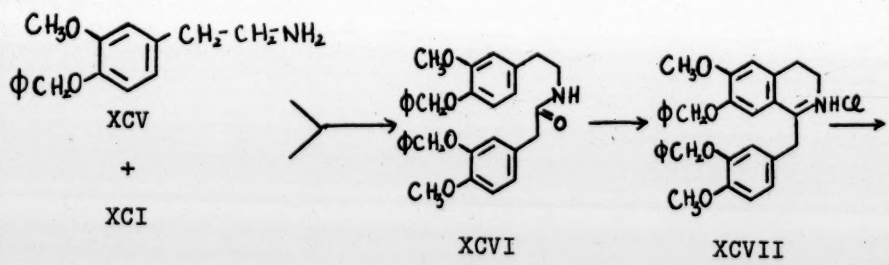
LXXXVIII LXXXIX XC



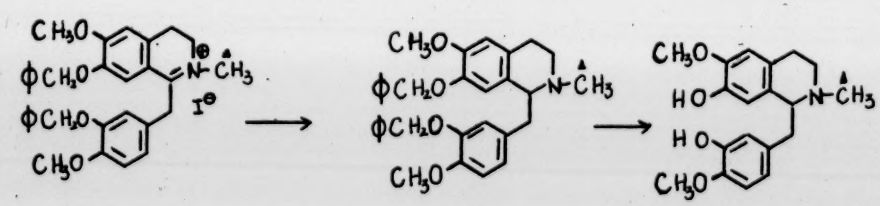
XCI



XCII XCIII XCIV

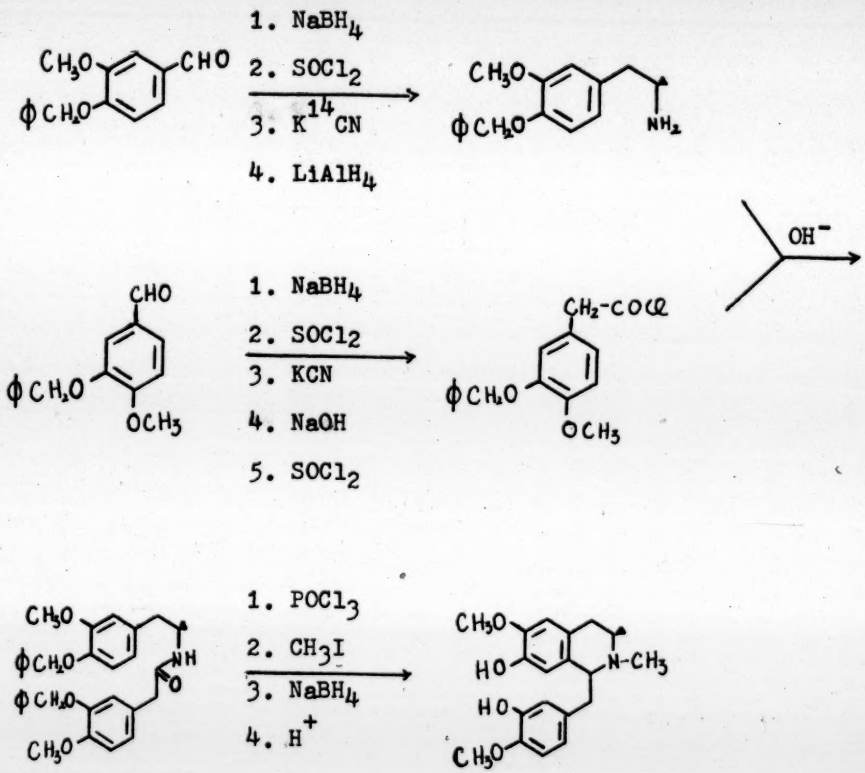


XCV + XCI XCVI XCVII

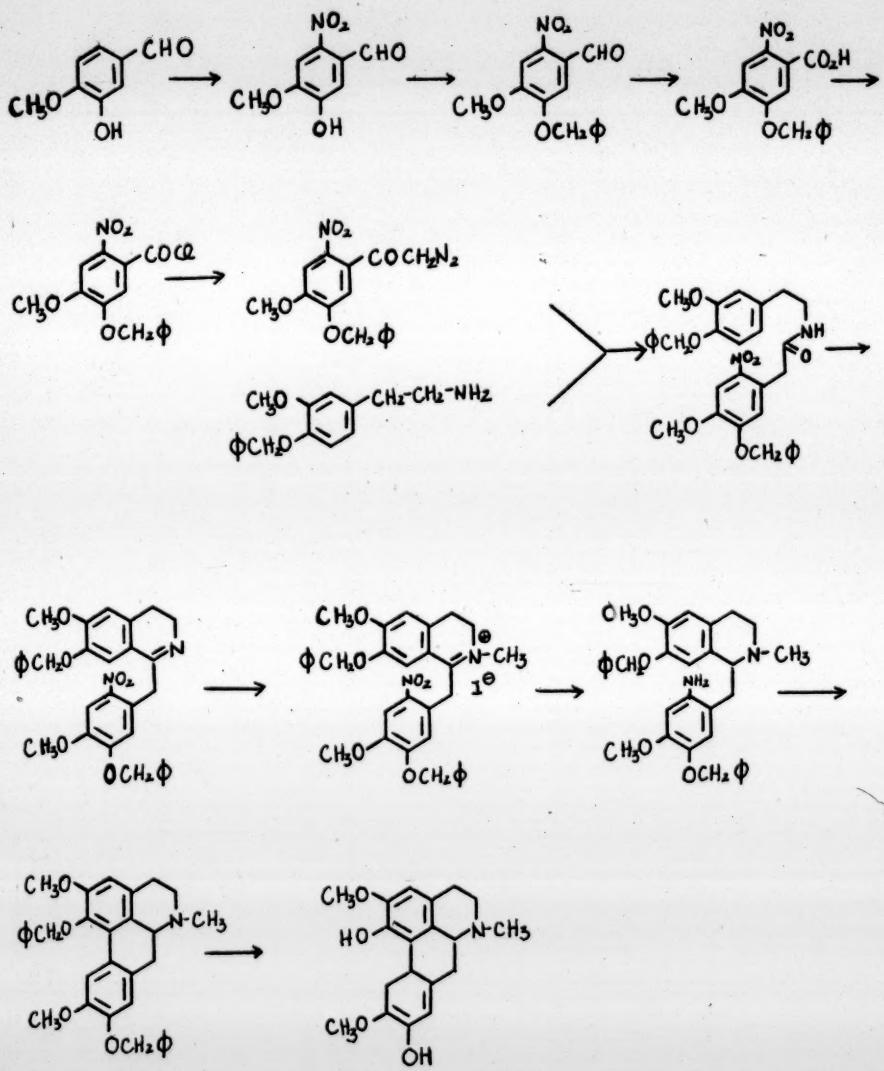


XCVIII XCIX C

Scheme V


1. NaBH4
2. SOCl2
3. K14CN
4. LiAlH4
OH⁻
1. NaBH4
2. SOCl2
3. KCN
4. NaOH
5. SOCl2
1. POCl3
2. CH3I
3. NaBH4
4. H+

Scheme VI



Scheme VII

second batch, plants were fed with (±)-reticuline-(N-$^{14}CH_3$), and reverse isotope dilution was performed with (+)-magnoflorine, (±)-isoboldine, isocorydine and tetrahydropalmatine. The third batch of plants were fed with (±)-reticuline-(N-$^{14}CH_3$), and boldine, glaucine together with oxycryptopine, sinomenine hydrochloride and berberine sulfate were added as diluents.

4. Isolation of Alkaloids

The plants were cut with pruning shears and homogenized with methanol. Alkaloids used for reverse isotope dilution were then added and the material extracted with methanol. Chlorophyll and other nonbasic material were removed by ethyl acetate extraction. The alkaloids in the acid extract were further separated into several fractions by liquid-liquid extraction based on their difference in physical properties. The extraction procedure for the first batch of plants is illustrated in Scheme VIII.

The extraction procedure for the second and third batches is the same and is illustrated in Scheme IX.

The alkaloid fractions were subjected to column chromatography on silica gel or aluminum oxide or preparative thin layer chromatography, and the compounds obtained in this way were purified by crystallization from a suitable solvent to constant radioactivity.

In the first part of our study, we were only able to isolate about 10 mg. of isoboldine. It was converted to

glaucine methiodide by reaction with diazomethane and iodomethane. In the second feeding experiment, isoboldine was isolated and purified as such.

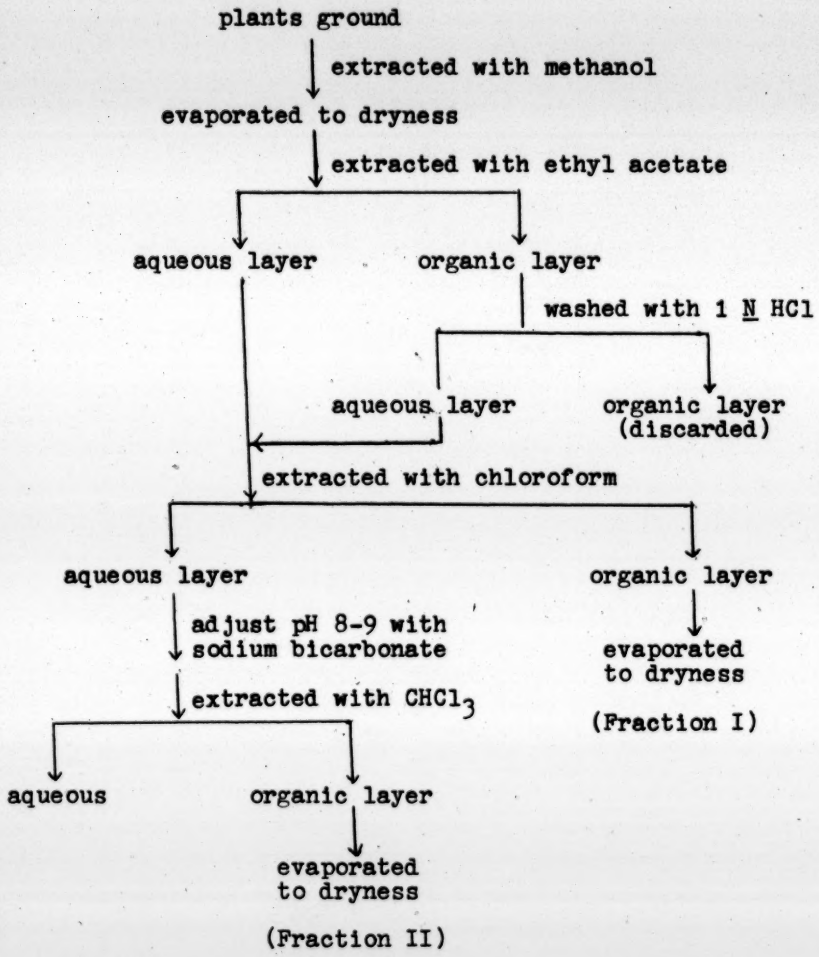
Magnoflorine is a quaternary amine and was found in the aqueous layer as the salt. Treatment with ammonium reineckate precipitated the alkaloid as the reineckate salt, which was dissolved in acetone. It was converted to water soluble salt by means of silver sulfate and purified as the slightly soluble iodide.

Glaucine was converted to the methiodide derivative and crystallized from a mixture of methanol and water.

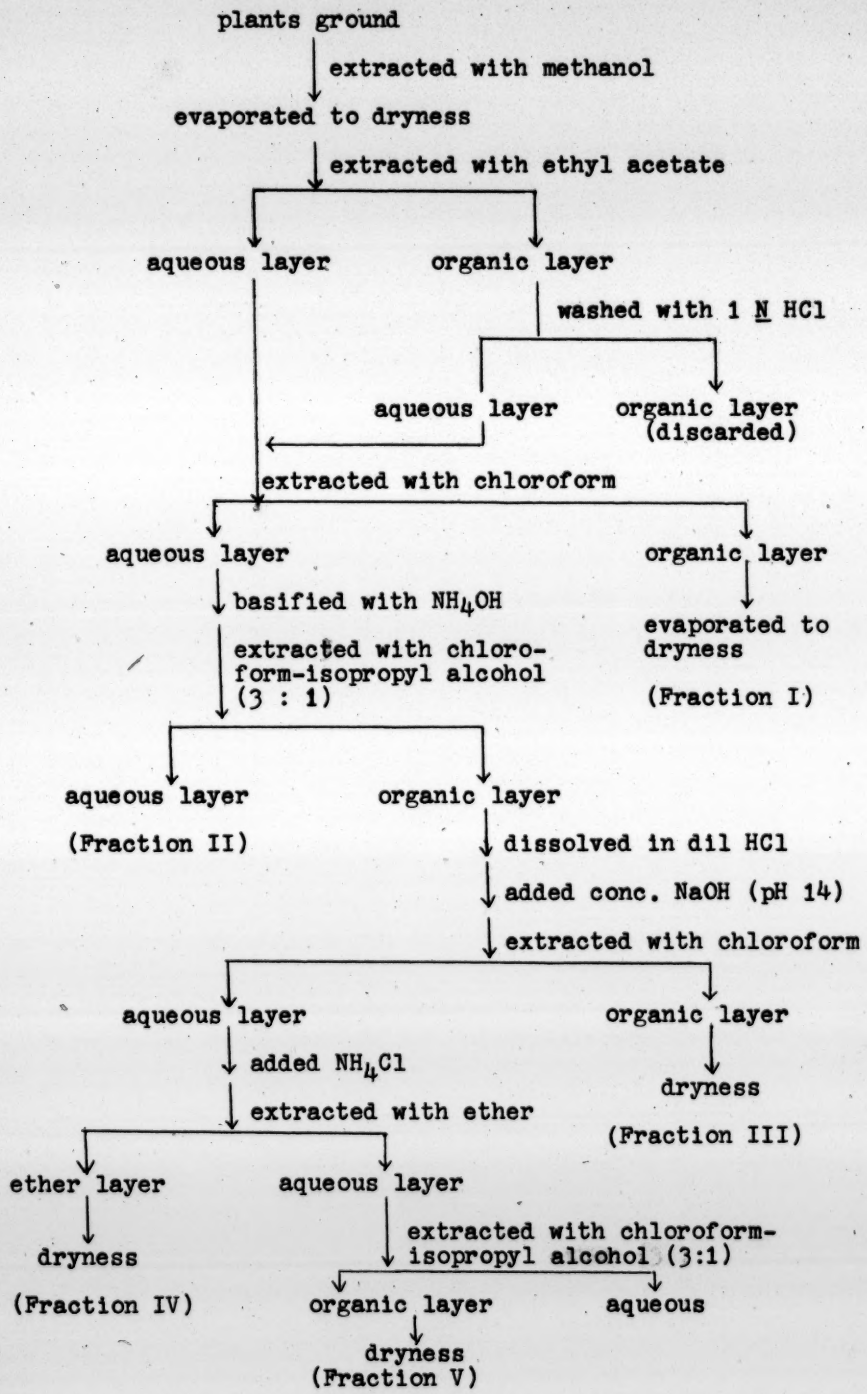
5. Determination of Radioactivity

The radioactivity was determined by liquid scintillation counting in a nonpolar (toluene-type) or a polar (dioxane-type) scintillation fluid depending on the solubility of the substance. Toluene-^{14}C was used as internal standard. Only isoboldine showed a significant radioactivity. The isoboldine isolated from plants fed with (±)-reticuline-(N-$^{14}CH_3$) had specific activity of 900 disintegrations/min./mg. or 132.6 μCi/mole (0.084% incorporation). The isoboldine isolated from plants fed with (±)-reticuline-(3-^{14}C) had constant radioactivity of 634 disintegrations/min./mg. or 94.2 μCi/mole (0.073% incorporation).

6. Degradation


plants ground
extracted with methanol
evaporated to dryness
extracted with ethyl acetate
aqueous layer
organic layer
washed with 1 N HCl
aqueous layer
organic layer (discarded)
extracted with chloroform
aqueous layer
organic layer
adjust pH 8-9 with sodium bicarbonate
evaporated to dryness
(Fraction I)
extracted with $CHCl_3$
aqueous
organic layer
evaporated to dryness
(Fraction II)

Scheme VIII


plants ground
extracted with methanol
evaporated to dryness
extracted with ethyl acetate
aqueous layer
organic layer
washed with 1 N HCl
aqueous layer
organic layer (discarded)
extracted with chloroform
aqueous layer
organic layer
basified with NH4OH
extracted with chloro-form-isopropyl alcohol (3 : 1)
evaporated to dryness
(Fraction I)
aqueous layer
(Fraction II)
organic layer
dissolved in dil HCl
added conc. NaOH (pH 14)
extracted with chloroform
aqueous layer
organic layer
added NH4Cl
extracted with ether
dryness
(Fraction III)
ether layer
aqueous layer
dryness
(Fraction IV)
extracted with chloroform-isopropyl alcohol (3:1)
organic layer
aqueous
dryness
(Fraction V)

Scheme IX

To certify that no "scrambling" of the N-$^{14}CH_3$ label had occurred during the incorporation of reticuline-(N-$^{14}CH_3$), the isolated radioactive isoboldine was degraded by Zeisel demethylation method using the modified Clark apparatus.

Reaction with hydroiodic acid at 180° to 200° gave complete O-demethylation. When the temperature was raised to 360°, N-demethylation occurred. The iodomethane carrying the ^{14}C label was trapped as triethylmethylammonium iodide and purified by crystallization. Constant radioactivity was achieved after three recrystallizations, (1194 disintegrations/min./mg. or 131.2 μCi./mole).

7. Results and Discussion

The radioactivity of the purified alkaloids and the result of degradation are shown in Table I.

Our feeding experiments showed that reticuline-(N-$^{14}CH_3$) and reticuline-(3-^{14}C) were incorporated into isoboldine to an extent of approximately 0.08%, calculated on the basis of the amount of isoboldine added. This is a significant incorporation in view of the very small amount of isoboldine present in the opium poppy (15). Degradation of isoboldine from the reticuline-(N-$^{14}CH_3$) feeding showed that the location of the label was exactly as expected. These results provide strong support for the theory that aporphines with a 1,2,9,10-substitution pattern are biosynthesized by direct ortho-para coupling of

Table I.

Alkaloid isolated	Precursor and its specific activity (uCi./mg.)	Amount of precursor fed		No. of plants	Amount isotope diluent added (mg.)	Specific activity of alkaloid isolated		Incorporation (%)**	Degradation product and (%) of radioactivity retained
		uCi.	mg.			dpm.*/mg.	uCi./mole		
isoboldine	(±)-reticuline-(N-$^{14}CH_3$) 3.94	144.2	36.6	14	300	900	132.6	0.084	triethylmethylammonium iodide 98.9%
isoboldine	(±)-reticuline-(3-^{14}C) 4.036	80.31	19.9	18	200	634	94.2	0.073	—
isocorydine	(±)-reticuline-(N-$^{14}CH_3$) 3.94	144.2	36.6	14	250	6	0.93	0.00047	—
magnoflorine-iodide	(±)-reticuline-(N-$^{14}CH_3$) 3.94	144.2	36.6	14	400	9	1.92	0.0011	—
boldine	(±)-reticuline-(N-$^{14}CH_3$) 3.94	143.3	34.1	17	250	20	2.97	0.0016	—
glaucine	(±)-reticuline-(N-$^{14}CH_3$) 3.94	143.3	34.1	17	250	8	1.29	0.00067	—

*dpm. = disintegrations/min./mg.

**percentage incorporation = $\frac{\text{(specific activity of alkaloid isolated) x (amount diluent added)}}{\text{(specific activity of precursor) x (amount precursor fed)}} \times 100\%$

the corresponding benzyltetrahydroisoquinolines.

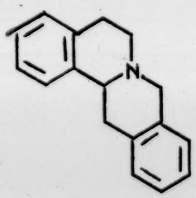
The fact that reticuline was not incorporated into magnoflorine indicated that aporphines substituted at positions 1, 2, 10 and 11 are not biosynthesized by the direct phenol coupling process in the opium poppy *via* reticuline. This is in contrast to the results obtained by Blaschke for bulbocapnine. However, our results are much more in conformity with the results of *in vitro* biogenetic type syntheses. It is possible that the same aporphine may be biosynthesized by different routes in different plants depending on the availability of precursors and enzymes. More work will be needed to elucidate the biosynthesis of magnoflorine and corytuberine in *Papaver somniferum*. Although the present investigation did not include the other possible biogenetic routes leading to corytuberine and magnoflorine, other benzyltetrahydroisoquinolines such as norlaudanosoline (XVI) and *N*-methylcoclaurine (LXI) may still be considered as potential precursors.

Our tracer studies showed no incorporation of reticuline into glaucine, isocorydine and boldine. From this, it would seem logical to conclude that the opium poppy (Indra variety) does not contain glaucine and boldine. No decision can be made, however, regarding the presence of isocorydine, since it, like corytuberine and magnoflorine, does not appear to be biosynthesized from reticuline in this plant.

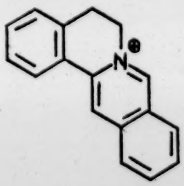
Part III. PROTOBERBERINES AND BERBERINES

A. Occurrence and Synthesis

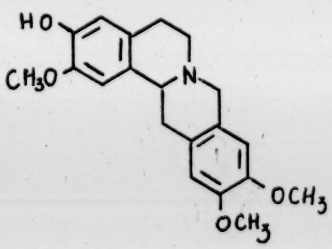
The protoberberines (CI) and their dehydro forms (berberines) (CII) occur in a wide variety of botanical families. The genera of the Papaveraceae contain mainly the tetrahydro bases (protoberberines), while the alkaloids in the Berberidaceae, Menispermaceae, Ranunculaceae, Rutaceae and Anonaceae exist mostly in the quaternary dehydro forms (berberines) (CII). The most common substitution pattern is 2,3,9,10. Those with 2,3,10,11 substitution pattern are normally called ψ-berberines or tetrahydro-ψ-berberines. So far, only three alkaloids isolated from nature belong to the tetrahydro-ψ-berberines, these are descretine (CIII) , coreximine (CIV) and xylopinine (CV). More rarely are substituents found in position 1 (e.g., capaurimine (CVI)) and in position 13 (e.g., ophiocarpine (CVII)) (43).



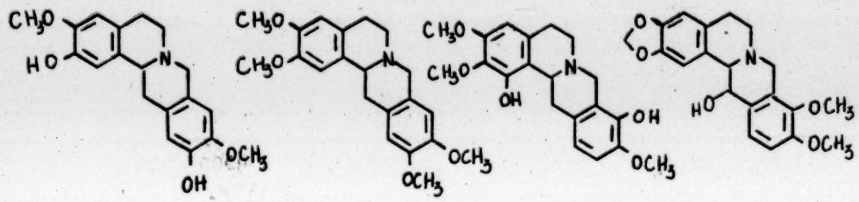
CI



CII

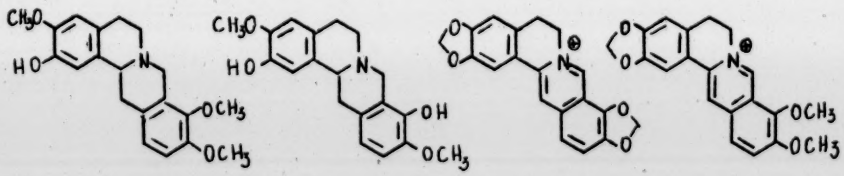


CIII



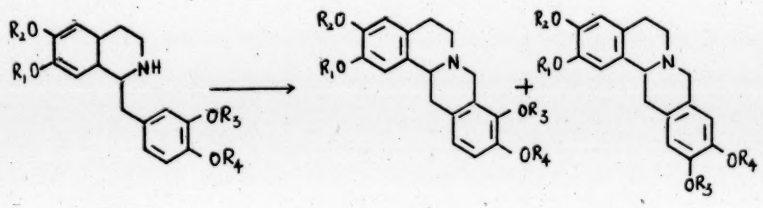
CIV CV CVI CVII

Several protoberberines and berberines have been found in the opium poppy. In 1962 Pfeifer and Teige (44) isolated a new opium alkaloid and named it somniferine. It was later identified as (-)-isocorypalmine (CVIII) by Pfeifer et al. (45) and by Brochmann-Hanssen et al. (46). (-)-Scoulerine (CIX) was isolated from the opium poppy by Brochmann-Hanssen et al. (47) in 1965. Using paper chromatography and paper electrophoresis, Hakim (2) in 1961 claimed the presence of coptisine (CX) in the opium poppy. He reported that tests for berberine (CXI) were negative. However, in a patented process for chromatographic separation, Ose (48) reported the isolation of berberine from opium poppy in appreciable quantities.



CVIII CIX CX CXI

Theoretically, protoberberines can be derived from benzyltetrahydroisoquinolines by condensation with formaldehyde. Such synthesis has been achieved in vitro. However, early attempts to synthesize protoberberines by this route failed (49), since the only product obtained was the isomer, tetrahydro-ψ-berberine (CXIV; R_1=R_2=CH_3; R_3+R_4=-CH_2-). Similar experiments (50) with the tetrahydroxy compound (CXII; R_1=R_2=R_3=R_4=OH), yielded a mixture of (CXIII; R_1=R_2=R_3=R_4=OH) and (CXIV; R_1=R_2=R_3=R_4=OH).



CXII CXIII CXIV

Recent work by Battersby et al. (51) using the dihydroxy-dimethoxy compound, norreticuline (CXII; R_2=R_4=CH_3; R_1=R_3=OH), gave also a mixture of the two isomers, the proportion of which depended on the pH of the reaction medium. Kametani (52) reported that the condensation of norreticuline with formaldehyde in methanol gave only coreximine (CIV). However, by repeating his work under the same condition, we obtained a mixture of coreximine (CIV) and scoulerine (CIX).

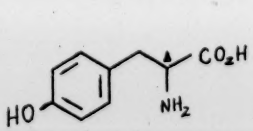
B. Biosynthetic Theories

Speculation on the biosynthesis of protoberberines

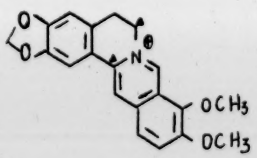
dates back to the beginning of the century. Most of the early proposals recognized the structural relationship of the protoberberines to the benzylisoquinoline bases. The additional carbon atom necessary for the conversion of the benzylisoquinoline system to that of the protoberberines was assumed by Sir Robert Robinson (3) to originate from formaldehyde or its biological equivalent.

These hypotheses have been of inestimable value for tracer experiments with living plants. Experiments on the biosynthesis of berberines and related alkaloids have had, in addition to these guidelines, the benefit of the knowledge gained from previous tracer studies on the biosynthesis of the benzylisoquinoline alkaloids which were known to be derived from two molecules of the aromatic amino acid, tyrosine.

Spenser and co-worker (53) investigated the biosynthesis of berberine and related alkaloids produced by Hydrastis canadensis L. They could show that DL-tyrosine-(2-^{14}C) (CXV) was efficiently incorporated into berberine (CXI). Controlled degradation of the labeled berberine established that the label was restricted to the carbon atoms in positions 6 and 14 as illustrated (CXI).



CXV



CXI

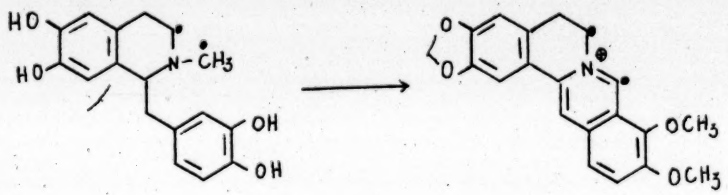
This indicated that two molecules of tyrosine participate in the biosynthesis of berberine, and that each unit of the amino acid is incorporated in a specific manner. Administration of 3,4-dihydroxy-2-phenylethylamine-(1-^{14}C) (XV) gave radioactive berberine in which the label was exclusively in the 6 position.

The foregoing results are in agreement with the idea proposed by Robinson and others and may be interpreted as follows: Tyrosine is hydroxylated by the action of tyrosinase to DOPA which undergoes decarboxylation to 3,4-dihydroxyphenethylamine and oxidative deamination to 3,4-dihydroxyphenylpyruvic acid. Condensation of these two products form the 1-benzyltetrahydroisoquinoline intermediate. Insertion of the C-1 unit of the berberine bridge would complete the skeleton of the protoberberine.

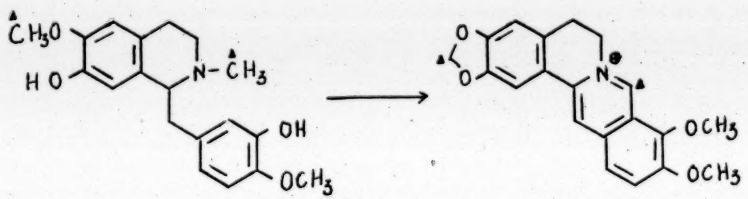
Experimental evidence for the nature of the benzylisoquinoline intermediates, as well as information regarding the formation of the berberine bridge have been provided by the research groups of Barton (54) and Battersby (55). The idea that the berberine bridge might be formed by the oxidative cyclization of an N-methyl group rather than by condensation of formaldehyde was conceived independently by both groups (56, 57) and proved to be correct by separate experiments.

Doubly labeled (±)-laudanosoline-(3-^{14}C; N-$^{14}CH_3$) (CXII) was administered to Berberis japonica and gave good incorporation into berberine (CXIII) with no scrambling of the labels (55).

Similarly, (±)-reticuline-(6-O-$^{14}CH_3$; N-$^{14}CH_3$) (CXIV) gave radioactive berberine (CXV) when fed to Hydrastis canadensis. Controlled degradation showed that the labels were in the expected positions (54).



CXII CXIII



CXIV CXV

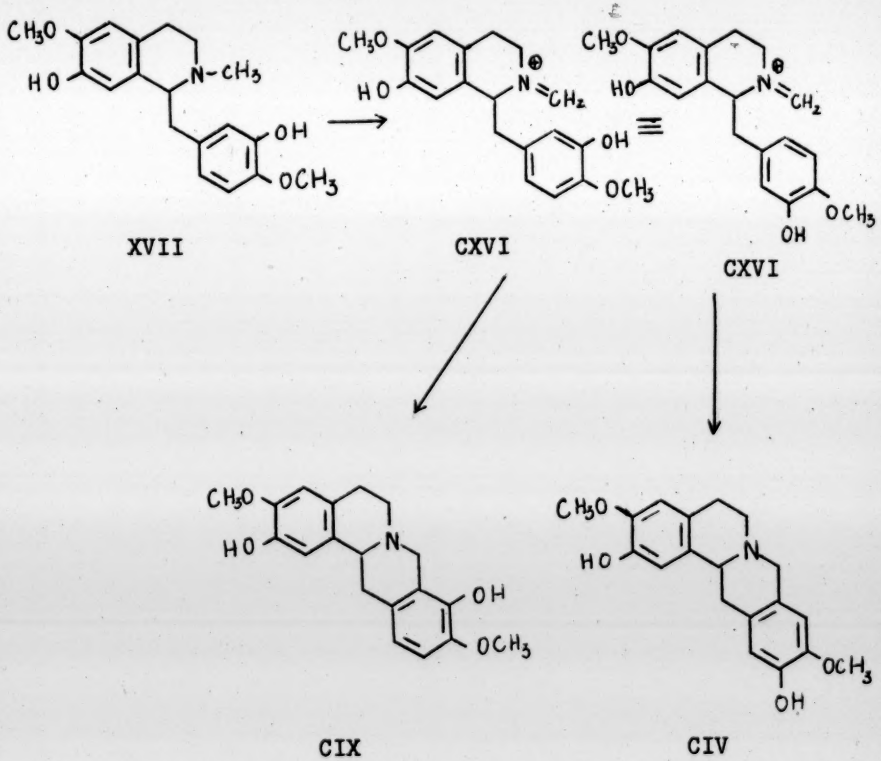
The steric requirement for the biosynthesis was studied by Barton et al. (58). Feeding of labeled (+)-reticuline and (-)-reticuline to Hydrastis canadensis in separate experiments resulted in the former being incorporated fifteen times more efficiently than the (-)-isomer. This indicated that (+)-reticuline is the true precursor.

In another experiment, the feeding of labeled (+)-reticuline to Hydrastis canadensis, gave a 0.035% incorporation into (-)-canadine which has the same absolute configuration as (+)-reticuline (59). Battersby et al. (60) have

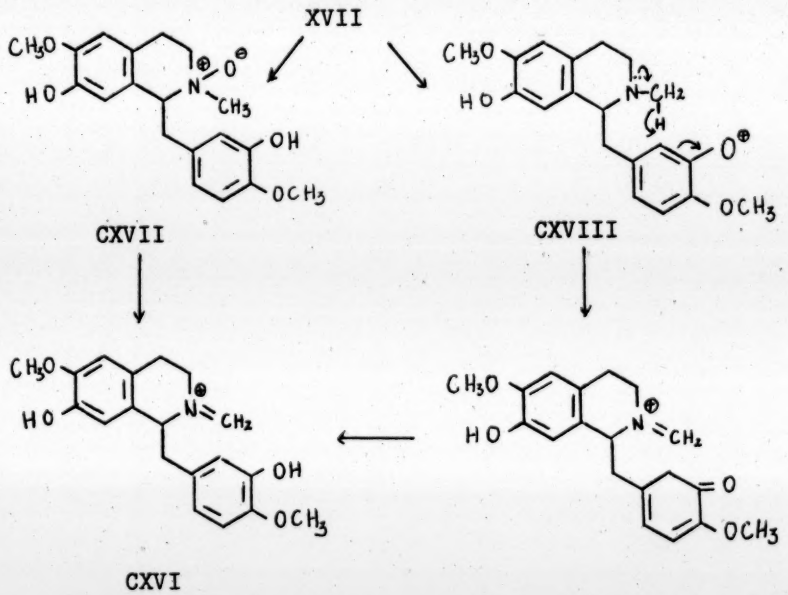
also demonstrated the incorporation of (+)-reticuline into (-)-scoulerine in the opium poppy.

These experiments give conclusive evidence that both protoberberines and berberines are biosynthesized from 1-benzylisoquinolines and that the berberine bridge is formed from the N-methyl carbon atom. Several possible mechanisms for the formation of the berberine bridge were proposed by both Barton et al. (58) and by Battersby, et al. (55)

Firstly, it may be envisaged that the formation of the berberine bridge involves the cyclization of a methylene-iminium salt (CXVI) in the sequence of (XVII → CXVI → CIV or CIX).

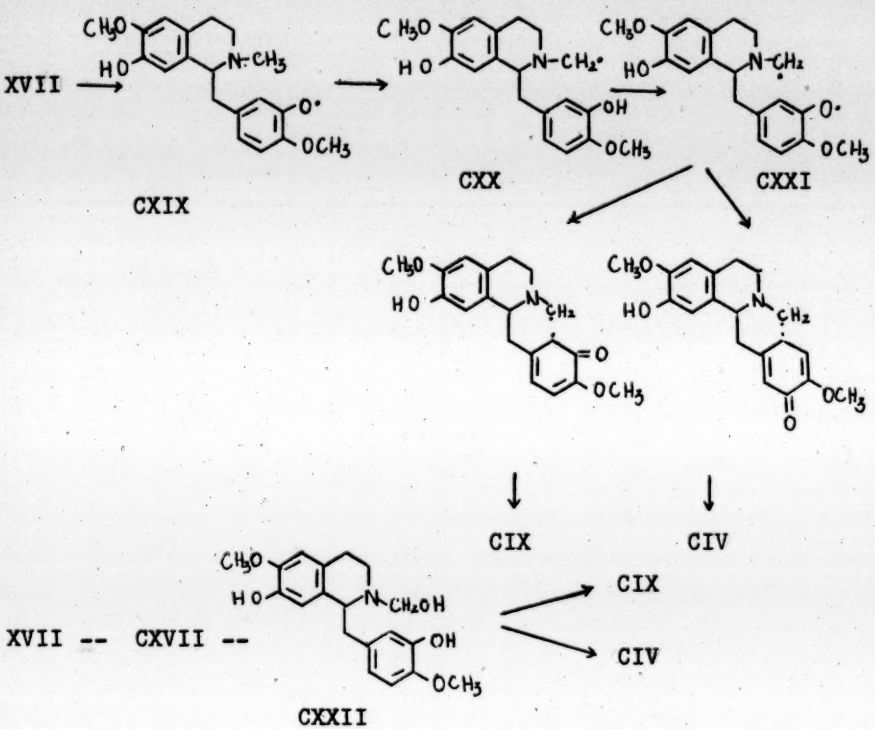


The iminium salt (CXVI) may be obtained by direct dehydrogenation of an N-methyl group or via the corresponding N-oxide (CXVII). It is also possible to visualize a two electron oxidation of a phenol to a phenoxonium ion (CXVIII) from which the iminium salt (CXVI) can be derived by intramolecular hydride transfer.



Secondly, the oxidation of the phenolic hydroxyl of (XVII) to a phenolate radical (CXIX) followed by hydrogen transfer from the N-methyl group, gives (CXX). Further phenolate oxidation to the biradical (CXXI) would then be followed by the ring closure reaction.

Thirdly, the formation of the N-oxide (CXVII) followed by rearrangement to the carbinolamine (CXXII) and ring closure reaction, gives the tetrahydroprotoberberine.



It would be difficult to distinguish between the above three mechanisms.

C. Potential Protoberberine Alkaloids in the Opium Poppy and their Relationship to Reticuline

From a mechanistic point of view, two products can be formed during the cyclization of a 1-benzyltetrahydroisoquinoline. The coupling at a position ortho to the hydroxyl group gives a tetrahydroberberine, whereas coupling at the para position will give a tetrahydro-γ-berberine. (-)-Scoulerine has been shown to be derived from (+)-reticuline in the opium poppy (60). However, its isomer, coreximine (CIV),

has never been isolated from this plant. The alkaloid coreximine was first isolated from Dicentra eximia by Manske(61). It is levoratory and its absolute configuration is identical with that of (-)-scoulerine (51). Because of its relationship to (-)-scoulerine and (+)-reticuline, it would be logical to expect (-)-coreximine to be present in the opium poppy.

If feeding experiments with labeled reticuline to Papaver somniferum gives incorporation of radioactivity into coreximine, it may be concluded that coreximine is a normal opium alkaloid. Such results would also reveal the biosynthesis of tetrahydro-γ-berberines which has never been reported so far.

Intrigued by the contradictory reports of Hakim and Ose regarding the presence of berberine in opium, we decided to use the biosynthetic approach in an attempt to detect it and its tetrahydro form, canadine. These alkaloids are both derived from (+)-reticuline biosynthetically.

Since isocorypalmine (CVIII) (monomethyl scoulerine) has been isolated from opium, it would seem reasonable to expect that the fully methylated protoberberine, tetrahydropalmatine, also might be present. It was, therefore, included in our investigation.

D. Experimental Approach

1. Synthesis of Labeled Precursors

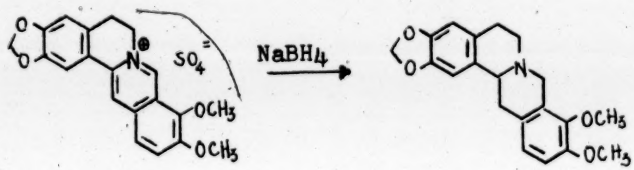
The radioactive precursors used for our feeding exper-

iments were (±)-reticuline-(3-^{14}C) and (±)-reticuline-(N-$^{14}CH_3$). Their syntheses have been described in Part II, (p. 26, 27).

2. Alkaloids for Reverse Isotope Dilution

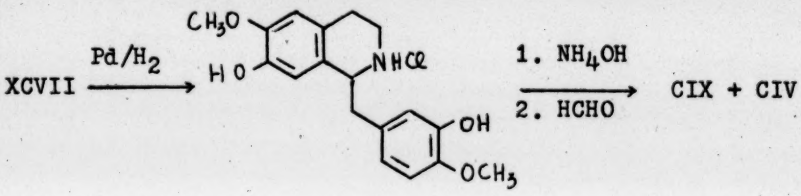
(a) Tetrahydropalmatine and berberine were purchased from Pierce Chemical Company.

(b) (±)-Canadine was synthesized by reduction of berberine sulfate with sodium borohydride in methanol.



CXI CXXIII

(c) (±)-Coreximine was synthesized by condensation of norreticuline (CXXIV) with formaldehyde (52). The early steps in the synthesis of norreticuline were the same as described for the synthesis of reticuline. The 1-(3-benzyloxy-4-methoxybenzyl)-3,4-dihydro-6-methoxy-7-benzyloxyisoquinoline hydrochloride (XCVII) so obtained, was subjected to catalytic hydrogenation and debenzylation at room temperature and atomospheric pressure. Norreticuline hydrochloride (CXXIV) was condensed with formaldehyde in methanol to give a mixture of (±)-scoulerine (CIX) and (±)-coreximine (CIV). The mixture was separated by column chromatography on neutral alumina with chloroform.



CXXIV

3. Feeding Procedure

The labeled precursors were administered to the plants as described in Part II (p. 28).

Two batches of plants were used. In the first batch, seventeen plants were fed with (±)-reticuline-(3-^{14}C), and the alkaloids (±)-canadine, (±)-coreximine and (±)-isoboldine were used as reverse isotope diluents.

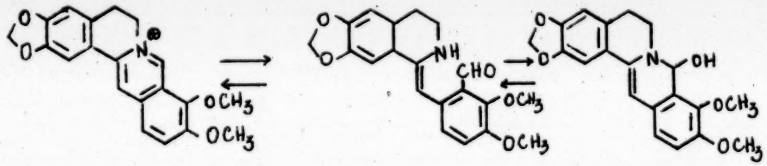
The second batch, consisting of fourteen plants, were fed with (±)-reticuline-(N-$^{14}CH_3$) and the protoberberine alkaloid, tetrahydropalmatine, together with the aporphines (±)-isoboldine, isocorydine and (+)-magnoflorine were added as isotope diluents.

4. Isolation of Alkaloids

The plants were ground with methanol in a Waring Blender, the alkaloids used for reverse isotope dilution were added and the material was extracted as described in Part II (p. 32). The methanol extract was concentrated to a small volume and the alkaloids separated into several fractions

by liquid-liquid extraction based on their physical properties (e.g., solubility, pKa, etc.). The extraction scheme of the first batch of plants is shown in Part II (p.34), Scheme VIII. The extraction scheme for the second batch of plants is shown in Part II (p. 35), Scheme IX.

Canadine was extracted from the acidic medium by chloroform. Berberine was obtained by chloroform extraction from a strongly alkaline medium (pH 14). Although berberine is a quaternary salt, it exists in three tautomeric forms (CXXV) (CXXVI) (CXXVII). In strongly basic medium the (CXXVI) and (CXXVII) forms predominate and can be extracted by ether or chloroform.



CXXV CXXVI CXXVII

The crude alkaloid fractions were subjected to column chromatography on silica gel or aluminum oxide. The compounds obtained in this way were purified by crystallization from a suitable solvent to constant radioactivity.

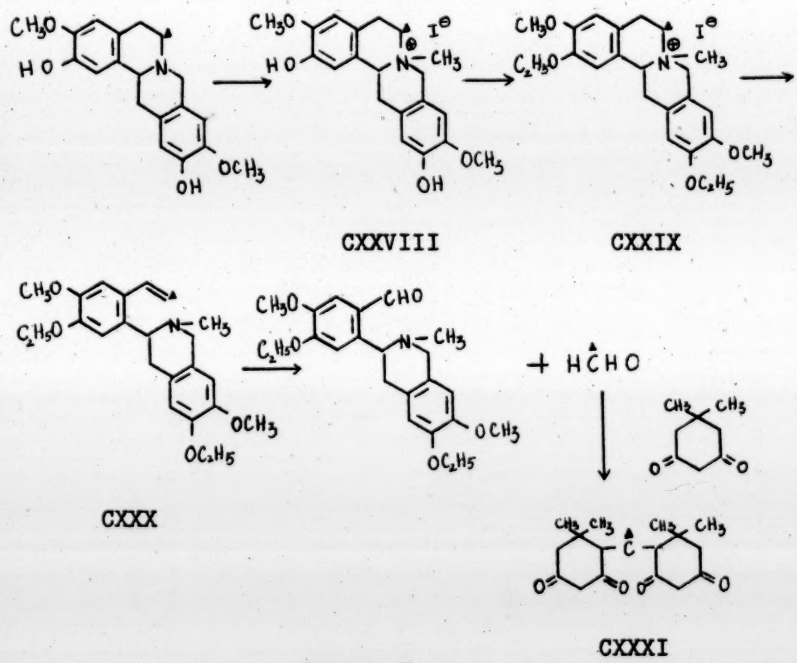
5. Determination of Radioactivity

The radioactivity of the purified alkaloids was measured as described in Part II (p. 33). Coreximine showed a significant radioactivity of 1480 disintegrations/min./mg.

or 220 μCi./mole. The percentage of incorporation was 0.174.

6. Degradation

To certify that no scrambling of labels occurred during the incorporation of (±)-reticuline-(3-^{14}C) into (±)-coreximine, the isolated radioactive (±)-coreximine was degraded as illustrated in Scheme X:



Scheme X

Coreximine was first reacted with methyl iodide to form the quaternary salt (CXXVIII), which was ethylated with ethyl iodide under alkaline condition. The resulting diethylcoreximine methyl iodide (CXXIX) underwent Hoffmann degradation to give O,O-diethylphellodendrine

methine (CXXX). The methine was subjected to ozonolysis, and the ozonide was cleaved by reduction with zinc powder in water to give formaldehyde which was separated by distillation. Formaldehyde carrying the radioactive label was trapped as the dimedone derivative (CXXXI) and recrystallized three times, yielding a constant radioactivity of 1423 disintegrations/min./mg. or 196 μCi./mole.

7. Results and Discussion

The radioactivity of the isolated alkaloids and the results of the degradation are shown in Table II.

Our feeding experiments with (±)-reticuline-(3-^{14}C) followed by isotope dilution and isolation gave radioactive coreximine with a specific activity of about 1480 disintegrations/min./mg. corresponding to an incorporation of 0.17% calculated on the basis of the amount of coreximine added. Controlled degradation showed that at least 90% of the radioactivity was located at position 6 as expected. The slightly low value found in the dimedone derivative is a result of dilution by non-radioactive formaldehyde arising from an N-methyl group during the degradation process. Battersby *et al.* (62) have recently reported a very similar case. They degraded a compound called autumnaline (CXXXII) which supposedly had a radioactive label located at position 3, however, the degradation result showed that the resulting dimedone had only 85% of the total radioactivity.

Table II.

Alkaloid isolated	Precursor and its specific activity (uCi./mg.)	Amount of precursor fed		No. of plants	Amount isotope diluent added (mg.)	Specific activity of alkaloid isolated		Incorporation (%)**	Degradation product and (%) of radioactivity retained
		uCi.	mg.			dpm.*/mg.	uCi./mole		
corexi-mine	(±)-reticuline-(3-^{14}CH$_3$) 4.036	80.31	19.9	18	210	1480	220	0.174	formalde-hyde-dime-don 90%
canadine	(±)-reticuline-(3-^{14}C) 4.036	80.31	19.9	18	200	10	1.54	0.0011	—
tetrahy-dropal-matine	(±)-reticuline-(N-^{14}CH$_3$) 3.94	144.2	36.6	14	250	11	1.77	0.00085	—
berberine sulfate	(±)-reticuline-(N-^{14}CH$_3$) 3.94	134.3	34.1	17	250	7	1.22	0.00059	—

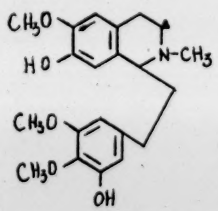
*dpm. = disintegrations/min./mg.

**percentage incorporation = $\frac{\text{(specific activity of alkaloid isolated) x (amount diluent added)}}{\text{(specific activity of precursor) x (amount precursor fed)}}$ x 100%

Our experiments indicate that the opium poppy is capable of synthesizing coreximine, therefore, coreximine should be considered a normal member of the opium alkaloids. Our results also support the idea that tetrahydro-ψ-berberines are derived biosynthetically in the same manner as the tetrahydroberberines.

The incorporation of radioactivity into berberine was negligible. This indicated the absence of berberine in the opium poppy. This result was further substantiated by the finding that canadine was also non-radioactive, since canadine presumably is the precursor of berberine. Therefore, we concluded that both canadine and berberine are not normally present in the opium poppy, at least not in the Noordster and Indra varieties used in our experiments. Our results are in agreement with the work of Hakim et al. who were also unable to detect berberine in the opium poppy, but contradict the report of Ose et al.

Our isotope dilution study of tetrahydropalmatine also gave a negative result. This alkaloid is, therefore, not a normal member of the opium alkaloids.

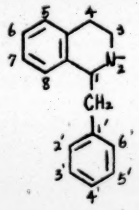


CXXXII

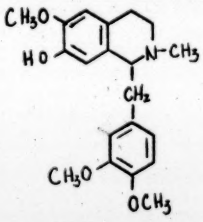
Part IV. BENZYLTETRAHYDROISOQUINOLINES

A. Occurrence

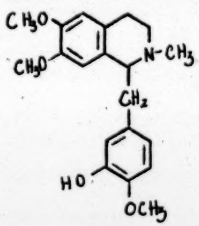
1-Benzyltetrahydroisoquinoline alkaloids have the structural skeleton and numbering system shown in (CXXXIII).



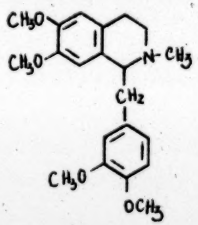
CXXXIII



CXXXIV



CXXXV



CXXXVI

A number of alkaloids of this group have been isolated from the opium poppy. In 1870, Hesse (63) isolated (+)-codamine (CXXXIV) and (±)-laudanine (CXXXV) from an opium extract, each in amounts of 0.003-0.005%. The next year, (+)-laudanosine (CXXXVI) was isolated. During the isolation of laudanine, which is optically inactive, Hesse found an optically active compound, which he named laudanidine (64). It was later found to be the levo-enantiomer corresponding to laudanine. In 1964, a diphenolic compound, (±)-reticuline (XVII) was isolated by Brochmann-Hanssen et al. (11) and the following year they showed that the dextrorotatory isomer is present in a larger amount than the levorotatory isomer (65).

B. Stereochemical Consideration

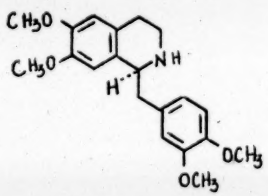
The stereochemistry of the 1-benzyltetrahydroisoquinolines has been studied by Corrodi and Hardegger (66). They determined the absolute configuration of (-)-norlaudanosine (CXXXVII) by converting it to N-β-carboxyethyl-L-aspartic acid (CXXXVIII) of known absolute configuration. By correlation with (-)-norlaudanosine, the absolute configuration of 1-benzyltetrahydroisoquinolines isolated from the opium poppy are as follows.

D(-) and L(+) reticuline (with an excess of L(+)-reticuline)

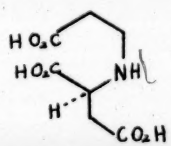
D(-) and L(+) laudanidine (with an excess of D(-)-laudanidine)

L(+) codamine

L(+) laudanosine



CXXXVII



CXXXVIII

C. Biosynthetic Theory

Compared with other groups, the 1-benzyltetrahydroisoquinolines have a simple structure. However, they are playing important roles in the biosynthetic pathways since they are the fundamental building blocks for other opium alkaloids.

The alkaloid reticuline has proved to be the most im-

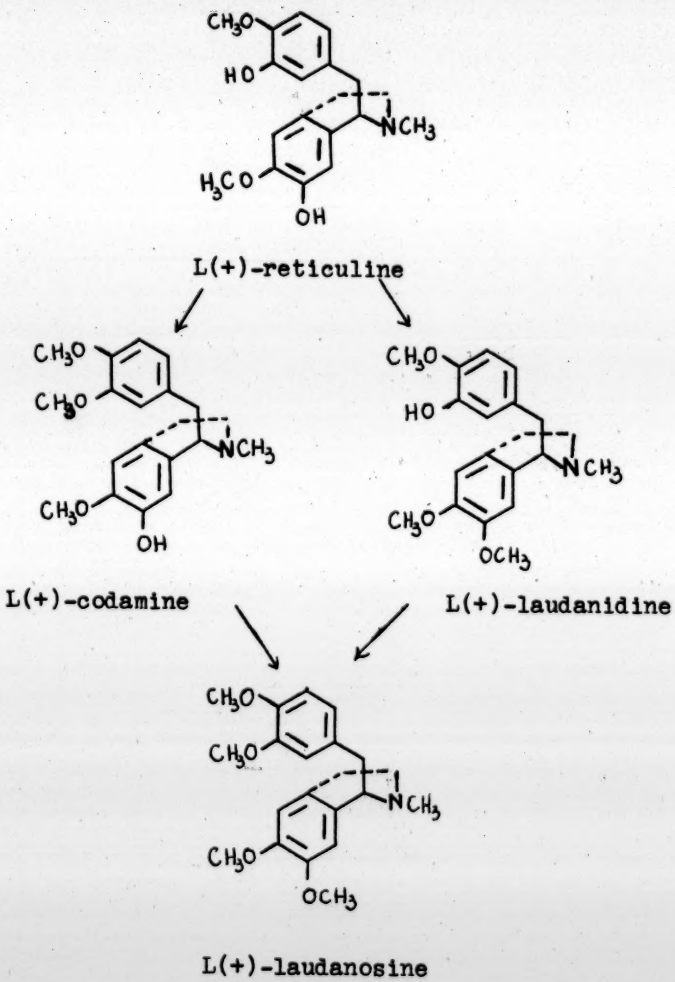
portant one from the biosynthetic point of view. Many of the biosynthetic pathways leading from reticuline have been discussed in the previous chapters.

Based on the accepted biosynthetic theory, the first product of the coupling of the two C_6-C_2 units derived from tyrosine is norlaudanosoline (XVI) which, according to Battersby *et al.* (67), has the L(-)-configuration. Methylation of norlaudanosoline at the nitrogen atom or the hydroxyl groups leads to various alkaloids.

The biosynthesis of laudanosine has never been studied. If (+)-reticuline can be assumed to be a precursor, there are two possible ways leading to (+)-laudanosine, namely *via* (+)-laudanidine, and *via* (+)-codamine. The possibility also exists that reticuline may not be directly involved, but that (+)-laudanosine may be formed from norlaudanosoline by random methylation. In that case, however, several additional diphenolic and monophenolic benzyltetrahydroisoquinolines should be present. There is no evidence that these exist in opium. The most likely pathways are the two illustrated in Scheme(XI). Since only L(+)-laudanosine has been found in the opium poppy, it should come from the L-series of codamine or laudanidine.

D. Experimental Approach

We decided to study the pathway involved in the formation of (+)-laudanosine from (+)-reticuline. Based on feeding experiments with radioactive (±)-codamine-(3-^{14}C) and (±)-laudanine-(*N*-$^{14}CH_3$), the incorporation of radioactivity



Scheme XI

into (+)-laudanosine would indicate the actual biosynthetic pathway. These experiments were performed in collaboration with Dr. Albert Leung.

1. Synthesis of Labeled Precursors

(a) The synthesis (68) of (±)-laudanine-(N-$^{14}CH_3$) followed a method similar to the synthesis of (±)-reticuline-(N-$^{14}CH_3$), which was discussed in Part II (p.26). The benzoyl group rather than benzyl group was used as the protecting group. The sequence of the synthesis is shown in Scheme XII.

(b) (±)-Codamine-(3-^{14}C) was synthesized by Dr. G. Zanati. The sequence of the synthesis is similar to Scheme VI.

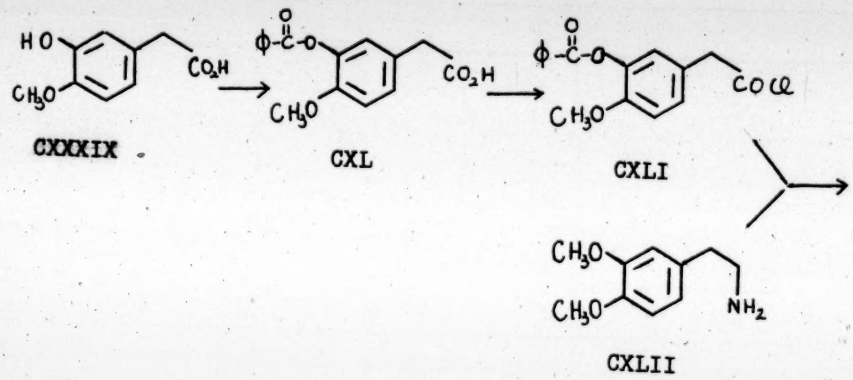
2. Alkaloid Used for Reverse Isotope Dilution

(±)-Laudanosine was obtained from Mallinchrodt Chemical Company.

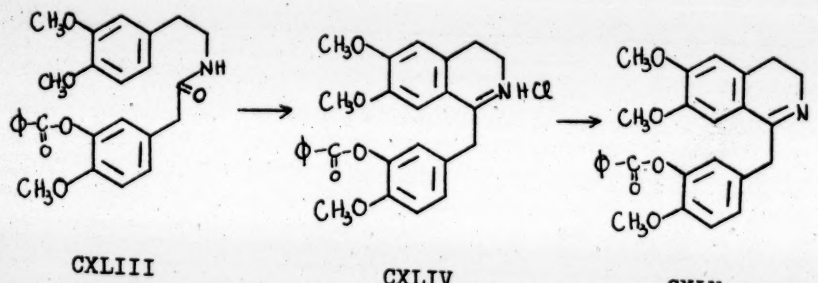
3. Feeding Procedure

The precursors were administered to Papaver somniferum (Indra variety) in a similar way as described in Part II (p. 28).

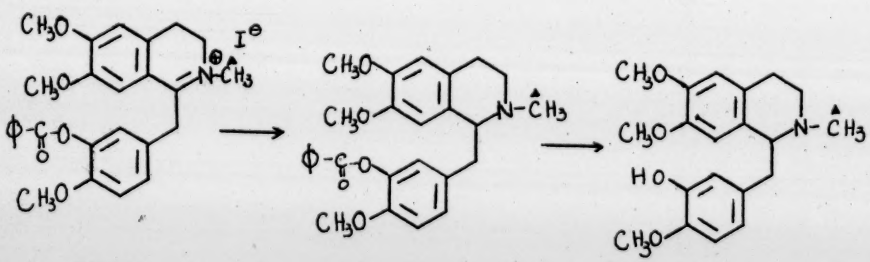
4. Isolation of Alkaloids



CXXXIX CXL CXLI CXLII



CXLIII CXLIV CXLV



CXLVI CXLVII CXLVIII

Scheme XII

The plants were cut and homogenized with methanol in a Waring Blender, (±)-laudanosine (200 mg.) was added, and the material completely extracted with methanol. The nonbasic material was removed from the extract by partitioning it between acidified water and ethyl acetate. The acidic layer, containing the total alkaloids, was basified with sodium hydroxide to pH 14 and extracted with ether. The ether extracts were evaporated to give a residue, which was further purified by column chromatography.

5. Determination of Radioactivity

The radioactivity of the purified compounds was determined as described in Part II (p. 33).

6. Degradation

The radioactive laudanosine isolated from the (±)-laudanine-(N-$^{14}CH_3$) feeding, was degraded by the Zeisel demethylation method in a similar way as the degradation of isoboldine described on page 33.

7. Results and Discussion

The radioactivity of the purified alkaloids and the result of the degradation are shown in the Table III. The results of feeding experiments with (±)-laudanine-(N-$^{14}CH_3$) showed a good incorporation (about 0.155%) of radioactivity

Table III.

Alkaloid isolated	Precursor and its specific activity (uCi./mg.)	Amount of precursor fed		No. of plants	Amount isotope diluent added (mg.)	Specific activity of alkaloid isolated		Incorporation (%)**	Degradation product and (%) of radioactivity retained
		uCi.	mg.			dpm.*/mg.	uCi./mole		
laudanosine	(±)-laudanine-(N-$^{14}CH_3$) 4.43	44.3	10	7	200	768	123.5	0.155	triethylmethylammonium iodide 99%
laudanosine	(±)-codamine-(3-^{14}C) 1.34	10.96	8.2	5	200	44	7.07	0.036	—

*dpm.=disintegrations/min./mg.

$$**\text{percentage incorporation} = \frac{(\text{specific activity of alkaloid isolated}) \times (\text{amount diluent added})}{(\text{specific activity of precursor}) \times (\text{amount precursor fed})} \times 100\%$$

into laudanosine based on the amount of laudanosine added.

Controlled degradation showed that all radioactivity was located at the N-methyl carbon atom as expected. On the other hand the feeding experiment with (±)-codamine-(3-^{14}C) showed only negligible incorporation (0.035%) of radioactivity. Therefore, the major pathway leading from (+)-reticuline to (+)-laudanosine is *via* (+)-laudanidine. Biosynthesis *via* (+)-codamine is only a minor pathway.

These results imply that methylation in the plant cells is not at random but proceeds in a specific order. The methylation process is usually for the protection of certain functional groups, so as to stabilize the molecule by decreasing their chemical reactivity and, in some cases, to direct the molecule to a certain desired reaction. Thus, the methylation of reticuline will prevent further ring cyclization into various groups of alkaloids.

As far as we know, methylation of the amine nitrogen and of the phenolic hydroxy groups involve a transmethylation process, in which "active" methyl groups of methionine, betaine, etc. take part.

There must be some specific enzymes, such as methyltransferase, involved in the methylation process. The nature of these enzymes must be the decisive factor controlling the methylation of (+)-reticuline to (+)-laudanosine. However, very little is known about these enzyme systems.

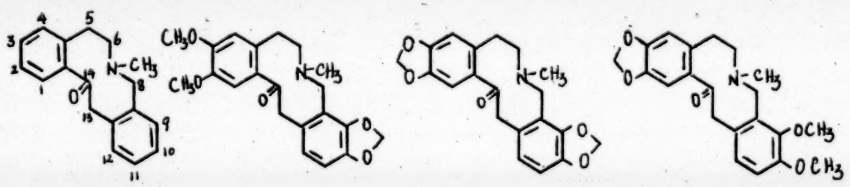
Our results are supported by the fact that there is an excess of D(-)-laudanidine in opium. This may possibly be explained in such a way that, although both D- and L-lau-

danidine are present in opium, methylation of L-laudanidine to L-laudanosine may leave an excess of D-laudanidine.

Part V. PROTOPINE ALKALOIDS

A. Occurrence

The protopine alkaloids (CXLIX) are characterized by the presence of a ten-membered N-hetero-ring containing a carbonyl group. They are widely distributed in Papaveraceae plants. Several protopines have been isolated from the opium poppy. Smith (69) in 1867, isolated cryptopine (CL) from the thebaine fraction of opium alkaloids. Protopine (CLI) was isolated from opium by Hesse (70) in 1871. Another alkaloid allocryptopine (CLII) was isolated by Brochmann-Hanssen et al. (71) in 1966.

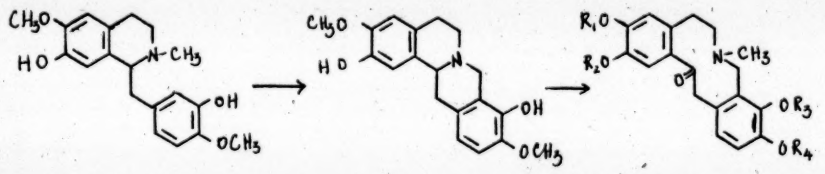


CXLIX CL CLI CLII

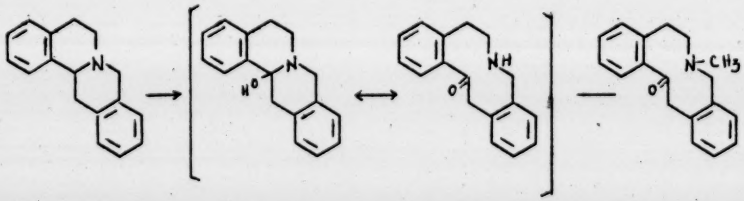
B. Biosynthetic Theory

The biosynthesis of protopines was studied by Barton et al. (8) and by Battersby et al. (60). Dicentra spectabilis which had been fed (±)-reticuline-(N-$^{14}CH_3$), gave radioactive protopine, and degradation showed the activity to be located at position 8. Battersby et al. (60) fed scoulerine-(6-^{14}C-14-^{3}H) to Chelidonium majus, and the i-

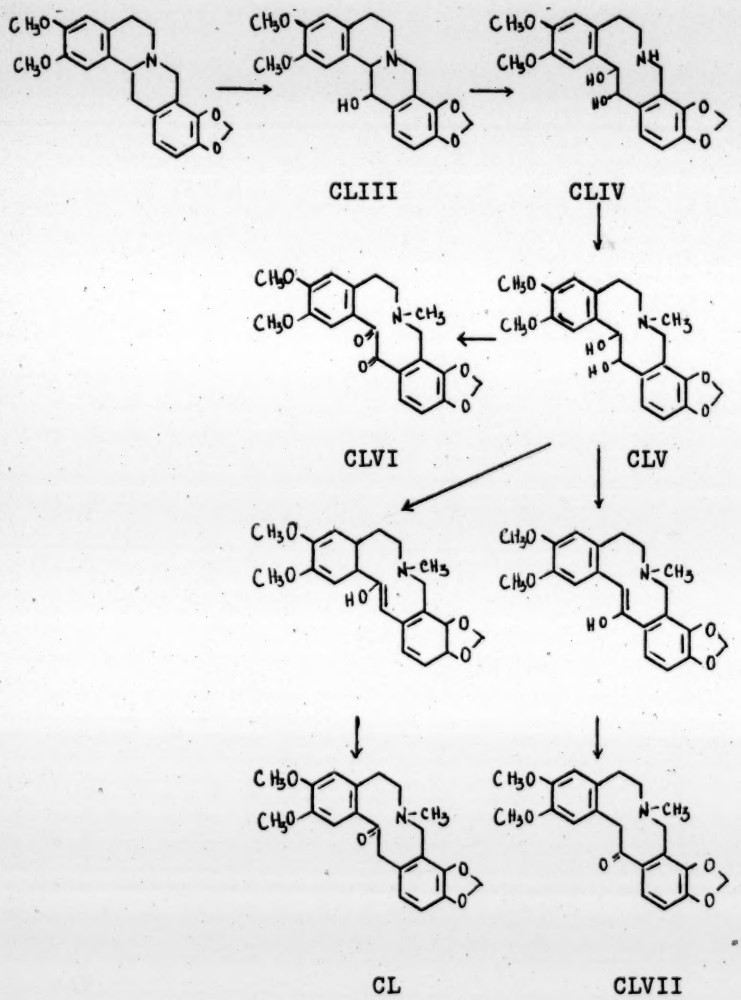
solated protopine had its radioactivity located at position 8. Furthermore, (-)-scoulerine was incorporated into protopine 20 times more efficiently than (+)-scoulerine. All these experimental evidences are in keeping with the postulated biosynthetic pathway as illustrated below:



The exact mechanism of the oxidative conversion of protoberberines to protopines is still not known. In his book on alkaloids, Manske (72) proposed two possible routes leading from protoberberine to protopine. The first envisages oxidation at the 14-position followed by N-methylation to yield protopine directly, as illustrated below:



The second pathway involves oxidation to (CLIII) and appropriate hydroxylation to (CLIV). N-Methylation and loss of water then leads to cryptopine (CL). This is illustrated as follows:



These two mechanisms have not yet been investigated by tracer techniques. However, the second pathway was favored since it readily accounted for the presence of alkaloids having the keto group in position 13. Such an alkaloid was reported by Manske (73) and called cryptocavine (CLVII).

However, later it was shown to be impure cryptopine. This removed any evidence which would tend to favor one mechanism over another. Recently, Brochmann-Hanssen et al. (74) isolated 13-oxycryptopine (CLVI) which would tend to support the pathway involving the diol (CLV) as an intermediate. The presence of 13-oxycryptopine (CLVI) may be explained as the oxidation product of the diol (CLV). Such oxidation of protopine can be readily achieved in vitro by means of mercuric acetate. Recently, Santavy et al. (75) also isolated an impure oxycryptopine from Papaver atlanticum. However, it is conceivable that 13-oxycryptopine may be an artifact produced by oxidation. This is always a possibility when the isolation is not carried on fresh plant material.

C. Experimental Approach

We decided to test whether the compound 13-oxycryptopine is actually present in the fresh opium poppy. Based on the biosynthetic theory, feeding experiment were performed with (±)-reticuline-(N-$^{14}CH_3$). 13-Oxycryptopine was added for reverse isotope dilution during extraction and subsequently isolated and purified. The incorporation of radioactivity into oxycryptopine would indicate its presence in the opium poppy.

1. Synthesis of Precursor

The synthesis of (±)-reticuline-(N-$^{14}CH_3$) has been described in Part II (p. 26).

2. Source of Alkaloid for Reverse Isotope Dilution

13-Oxycryptopine was obtained by oxidation of cryptopine with mercuric acetate as oxidizing agent (74).

3. Feeding Procedure

The plants were administed (±)-reticuline-(N-$^{14}CH_3$) as described in Part II (p. 28).

4. Isolation of Alkaloid

The isolation of 13-oxycryptopine was described in Part II , page 35, Scheme IX.

5. Determination of Radioactivity

The radioactivity of the purified oxycryptopine was measured as described in Part II (p. 33).

6. Results and Discussion

The radioactivity of the isolated oxycryptopine is shown in Table IV.

These results, contrary to our expectation, showed only

Table IV.

Alkaloid isolated	Precursor and its specific activity (uCi./mg.)	Amount of precursor fed		No. of plants	Amount isotope diluent added (mg.)	Specific activity of alkaloid isolated		Incorporation (%)**	Degradation product and (%) of radioactivity retained
		uCi.	mg.			dpm.*/mg.	uCi./mole		
Oxycryptopine	(±)-reticuline-(N-$^{14}CH_3$) 3.94	13	34.1	17	250	10	1.74	0.0009	____

*dpm.=disintegrations/min./mg.

**percentage incorporation=$\frac{\text{(specific activity of alkaloid isolated) x (amount diluent added)}}{\text{(specific activity of precursor) x (amount precursor fed)}}$ x 100%

negligible incorporation of radioactivity from (±)-reticuline-(N-$^{14}CH_3$) into oxycryptopine. Therefore, oxycryptopine might not be a normal member of the opium alkaloids. The isolated oxycryptopine might be an artifact. Again, this will remove the circumstantial evidence which would tend to favor one mechanism over another. Tracer studies based on feeding of various possible intermediate will throw some light on this problem.

Part VI. HYDROPHENANTHRENE ALKALOIDS

A. Occurrence

The hydrophenanthrenes, morphine, codeine and thebaine, represent three of the major alkaloid constituents of opium.

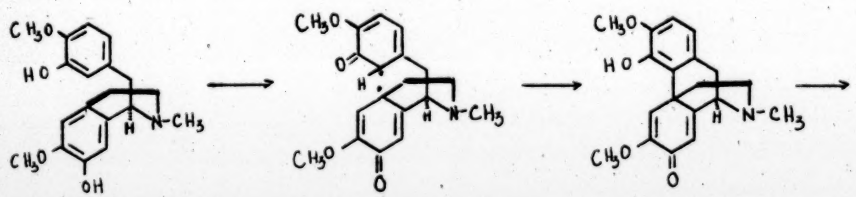
Previously, the hydrophenanthrenes have only been found in a few species of the genus Papaver. However, it has recently been reported that they are also present in three genera of the Menispermaceae, namely Menisperum, Sinomenium and Stephania (43).

Several new hydrophenanthrenes were isolated from the opium poppy during the past few years, such as 16-hydroxythebaine (76) and salutaridine (CLIX) (74). Although they are present in low concentration some of them give important support to modern theory for the biosynthesis of hydrophenanthrenes.

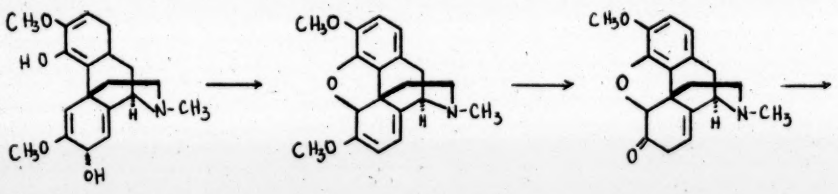
B. Stereochemical Considerations and Biosynthetic Theory

The biosynthesis of hydrophenanthrenes has posed one of the most interesting problems in the past several years. It is now generally agreed that thebaine is biosynthesized from (-)-reticuline (CLVIII) through oxidative coupling to the dienone salutaridine (CLIX). It is further reduced to salutaridinol (CLX) which undergoes rearrangement to thebaine (CLXI) (9, 56, 77). It has also been shown that thebaine (CLXI) is the first major hydrophenanthrene alkaloid (78) produced in

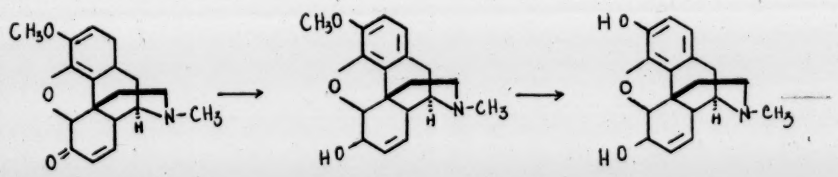
opium. It is converted to codeine (CLXIV) which in turn is demethylated to morphine (CLXV) (78, 79, 80). The biosynthetic pathways leading from (-)-reticuline to morphine (CLXV) is illustrated in Scheme XIII.



CLVIII CLIX



CLX CLXI CLXII

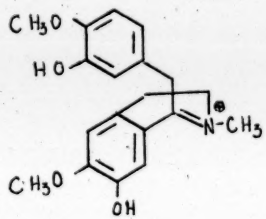


CLXIII CLXIV CLXV

Scheme XIII

Again, the importance of reticuline as a key precursor in the biosynthetic pathways is demonstrated. A rather im-

portant feature of reticuline is its stereochemistry. The particular form of reticuline shown in formula (CLVIII) is the levoratory enantiomer which has the same configuration as thebaine (CLXI). It follows that (-)-reticuline and not (+)-reticuline should be the precursor of thebaine. Although (+)-reticuline was also shown to be incorporated into morphine, Battersby (67, 81) proposed that an oxidation-reduction equilibrium exists between reticuline and 1,2-dehydroreticuline (CLXVI). Feeding experiments showed that labeled 1,2-dehydroreticuline was incorporated into morphine.

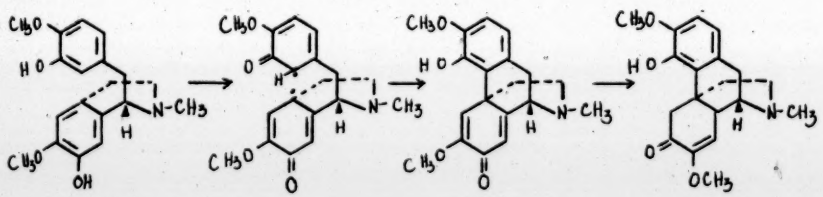


CLXVI

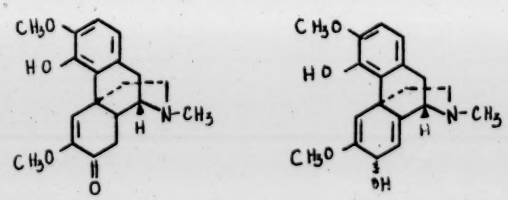
As shown in the pathways illustrated in Scheme XIII, oxidative phenol coupling of (-)-reticuline (CLVIII) to salutaridine (CLIX) is the step responsible for the formation of the hydrophenanthrene skeleton from a benzylisoquinoline. Barton and Kirby (82) have recently shown that sinomenine (CLXIX) in Sinomenium acutum is derived from (+)-reticuline (CLXVII) via sinoacutine (CLXVIII), an optical isomer of salutaridine (CLIX). The sequence of the biosynthesis is as illustrated (CLXVII → CLXVIII → CLXIX).

Feeding experiments have shown that (+)-reticuline and sinoacutine are incorporated well into sinomenine, thus es-

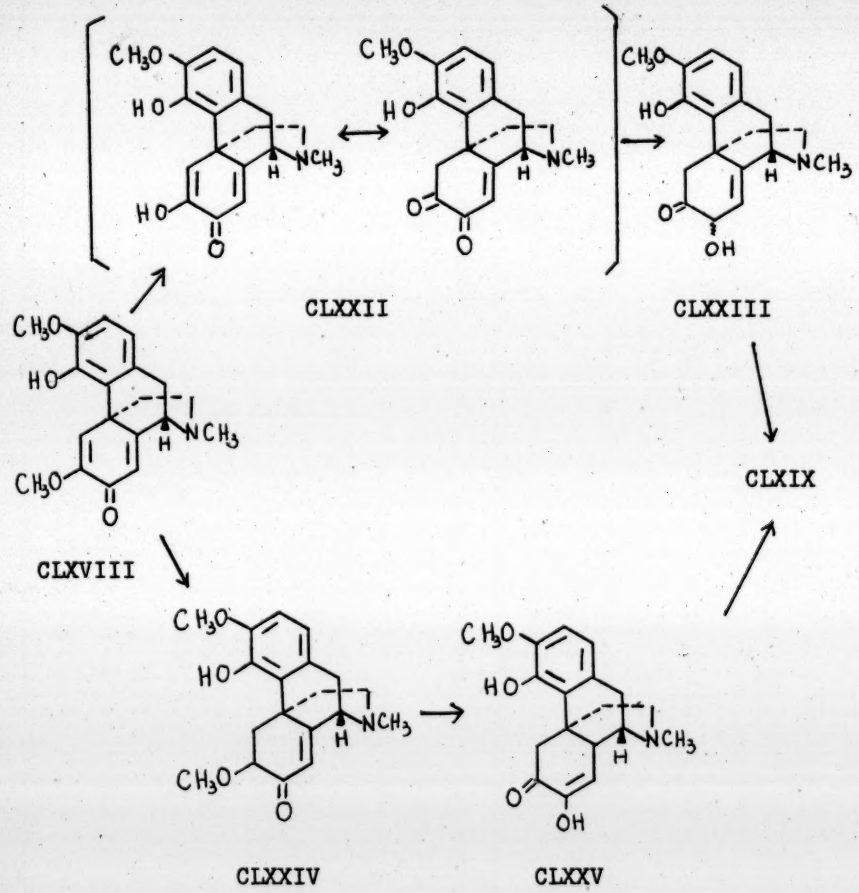
tablishing the correctness of the sequence (CLXVII → CLXVIII → CLXIX). The final details of the biosynthetic pathway from sinoacutine (CLXVIII) to sinomenine (CLXIX) are still not known. Several possible mechanisms were proposed by Barton (82) who showed that isosinomenine (CLXX) and sinoacutinol (CLXXI) were not on the biosynthetic pathway. Two remaining possibilities are illustrated in Scheme XIV. The first possibility is the hydrolysis of 6-methyl group of sinoacutine (CLXVIII) to give (CLXXII) which by reduction would give (CLXXIII) and thence sinomenine. The second possibility is reduction of the vinyl ether of sinoacutine to (CLXXIV), and transformation by enolization and hydrolysis to demethylsinomenine (CLXXV).



CLXVII CLXVIII CLXIX



CLXX CLXXI



Scheme XIV

C. Experimental Approach

Since (+)-reticuline is present in the opium poppy, and oxidative enzymes are known to be rather nonspecific, there seems to be a possibility that sinoacutine and sinomenine might exist in Papaver somniferum L. Therefore, we fed plants with (±)-reticuline-(N-$^{14}CH_3$) and added sinomenine for reverse isotope dilution.

1. Synthesis of Radioactive Precursor

The synthesis of (±)-reticuline-(N-$^{14}CH_3$) has been described in Part II (p. 26).

2. Source of Alkaloid for Reverse Isotope Dilution

Sinomenine was purchased from Pierce Chemical Company.

3. Feeding Procedure

The labeled precursor was administered to the plants in the same way as described in Part II (p. 28).

4. Isolation of Alkaloid

The isolation of sinomenine was described in Part II (p. 32).

5. Determination of Radioactivity

The determination of radioactivity was described in Part II, page 33.

6. Results and Discussion

The radioactivity of sinomenine isolated from the plants fed with (±)-reticuline-(N-$^{14}CH_3$) is shown in Table V.

The result indicated that the opium poppy does not contain sinomenine. Although the necessary precursor (+)-reticuline is present in the opium poppy, the plants were not able to transform it into sinomenine. This may be due to the absence of the enzyme system required for the transformation. It might also be due to the stereospecificity of the oxidative enzymes involved. It would seem more preferable to use sinoacutine as an isotope diluent, but this alkaloid was not available at the time. Another feeding experiment of reticuline-(N-$^{14}CH_3$) with sinoacutine as a carrier will tell us whether the transformation of (+)-reticuline to sinomenine in opium was blocked at the sinoacutine stage (CLXVII → CLXVIII) or at the final sinomenine stage (CLXVIII → CLXIX).

Table V.

Alkaloid isolated	Precursor and its specific activity (uCi./mg.)	Amount of precursor fed		No. of plants	Amount isotope diluent added (mg.)	Specific activity of alkaloid isolated		Incorporation (%)**	Degradation product and (%) of radioactivity retained
		uCi.	mg.			dpm.*/mg.	uCi./mole		
sinomenine	(±)-reticuline-(N-$^{14}CH_3$) 3.94	134.3	34.1	17	250	11	1.64	0.0009	___

*dpm.=disintegrations/min./mg.

**percentage incorporation = $\frac{\text{(specific activity of alkaloid isolated) x (amount diluent added)}}{\text{(specific activity of precursor) x (amount precursor fed)}} \times 100\%$

EXPERIMENTAL

All melting points were determined with the Thomas-Hoover capillary melting point apparatus and were uncorrected. The infrared spectra were taken in potassium bromide with a Perkin-Elmer infrared spectrometer, model 137. The nuclear magnetic resonance spectra were determined at 60 MC, with the Varian Association high resolution spectrometer A 60-A. The samples were in deuterochloroform, tetramethylsilane being used as internal standard. Gas liquid chromatographic analyses were carried out with a F & M Biomedical gas chromatograph Model 400. Thin layer chromatographic (T.L.C.) analysis employed silica gel and neutral alumina plates. Potassium iodoplatinate spray and iodine vapor were used as visualizing agents. Ozonolysis was performed on a Towers Ozone Apparatus GE 150. The radioactivity was determined by liquid scintillation counting on a Packard (model 3003) Tri-Carb Liquid Scintillation Spectrometer. Two types of scintillation fluid were used; a nonpolar (toluene-type) and a polar (dioxane-type), depending on the solubility of the substance. Samples (0.5 to 2 mg.) were accurately weighed on a Cahn balance and dissolved in 0.1 ml. of methanol. Ten ml. of scintillation fluid was added. Toluene-^{14}C was used as internal standard. The efficiencies ranged from 82-84% for the nonpolar and from 77-79% for the polar fluid. The radioactivity was considered to be constant when two to three succeeding measurements differed by less than 5%. The constituents of the two types of scintillation fluid were as follows:

1. Scintillation fluid type I --- polar (dioxane-type) solvent.

   "Premix P." containing 98% of 2,5-diphenyloxazole (PPO) and 2% of 1,4-di(2-(2-phenyloxazolyl))-benzene (POPOP) (3.3 g.) and 52 g. of naphthalene were dissolved in a mixture of 250 ml. toluene, 250 ml. of dioxane and 150 ml. of absolute alcohol.

2. Scintillation fluid type II --- non-polar (toluene-type) solvent.

   Permablend I, containing 91% of PPO and 9% of dimethyl POPOP, (5.5 g.) was dissolved in toluene to make 1 liter solution.

A. Synthesis of Radioactive Precursors

1. Synthesis of (±)-Reticuline-(N-$^{14}CH_3$) (41) (see Scheme VI)

O-Benzylisovanillin (LXXXVI). Isovanillin (10.1 g.; 0.066 mole), 8.6 g. of benzyl chloride (0.068 mole) and 5 g. of potassium carbonate were suspended in 65 ml. of dimethylformamide. The mixture was heated in an oil bath at 100-110° for 2.5 hr., cooled and the resulting brown liquid was poured into 400 ml. of water. After standing in the refrigerator overnight, the crystals were collected by filtration and recrystallized from isopropyl ether, m.p. 60-62° (lit. (41) 62-63°) yield 14.4 g. (92%).

3-Benzyloxy-4-methoxybenzyl alcohol (LXXXVII). To a stirred solution of 10 g. (0.042 mole) of O-benzylisovanillin in a mixture of 80 ml. of methanol and 20 ml. of benzene was added 1.6 g. of sodium borohydride over a period of 1.5 hr. The temperature was raised to 40°, and a solution of 12 g. of potassium hydroxide in 20 ml. of water was added. After the mixture had been heated on a steam bath for 3.5 hr., the organic solvents were evaporated and the aqueous solution extracted several times with a mixture of ether and chloroform (4 : 1). The combined extracts were dried over anhydrous sodium sulfate and evaporated to dryness. The residue was crystallized from a mixture of benzene and light petroleum ether (b.p. 30-60°) to give 8 g. of 3-benzyloxy-4-methoxybenzyl alcohol (78% yield), m.p. 72-73° (lit. (41)

72-73°). The NMR spectrum showed a broad band centered at 1.7 p.p.m. which disappeared on addition of deuterium oxide, indicating the presence of hydroxyl function.

3-Benzyloxy-4-methoxybenzyl chloride (LXXXVIII). Thionyl chloride (12 g.) was added dropwise and with stirring over a period of 30 min. to a suspension of 8 g. (0.032 mole) of 3-benzyloxy-4-methoxybenzyl alcohol in 100 ml. of anhydrous ether. After stirring for 3 hr. the clear solution was evaporated to dryness. The residue was crystallized from light petroleum ether (b.p. 30-60°) to yield 5.28 g. (63%) of 3-benzyloxy-4-methoxybenzyl chloride, m.p. 73-74° (lit. (41) 72-73°).

The infrared spectrum exhibited bands at 2850, 1590, 1510, 1260, 1230, 1000, 750 and 685 cm^{-1}.

3-Benzyloxy-4-methoxyphenylacetonitrile (LXXXIX). 3-Benzyloxy-4-methoxybenzyl chloride (5.25 g.; 0.02 mole) was suspended in 35 ml. of dimethylformamide, and 1.96 g. of sodium cyanide (0.04 mole) was added. The suspension was stirred for 15 hr. at room temperature and 250 ml. of water was added to cause precipitation. The precipitate was collected and crystallized from a mixture of chloroform and petroleum ether (b.p. 30-60°) (1 : 1) to afford 3.3 g. (65%) of 3-benzyloxy-4-methoxyphenylacetonitrile, m.p. 79-81° (lit. (41) 79.5-80.5°).

The infrared spectrum was characterized by a band at 2250 cm^{-1} (-C≡N stretching band).

3-Benzyloxy-4-methoxyphenylacetic acid (XC). A solution of 3.6 g. of 3-benzyloxy-4-methoxyphenylacetonitrile (0.014 mole) in 40 ml. of aqueous potassium hydroxide (25% w./v.) was refluxed for 52 hr. After cooling, the solution was washed with ether (3 x 30 ml.) acidified with 1 N hydrochloric acid and extracted with ether. The ether extract was dried over anhydrous sodium sulfate and evaporated to dryness. The gummy residue was crystallized from ethyl acetate to give 3.2 g. of 3-benzyloxy-4-methoxyphenylacetic acid (84%), m.p. 126-127° (lit. (41) 127-128°).

The infrared spectrum was characterized by bands at 3500 cm^{-1} (-O-H stretching vibration), 1700 cm^{-1} (carbonyl stretching vibration) and other bands at 1400, 1270, 1140 and 1010 cm^{-1}.

O-Benzylvanillin (XCIII). Vanillin (22.8 g.; 0.15 mole), 21 g. of benzyl chloride (0.17 mole) and 11.4 g. of potassium carbonate were suspended in 180 ml. of dimethylformamide. The mixture was heated in an oil bath for 2.5 hr. After cooling, the reaction mixture was poured into 900 ml. of water producing an oily precipitate. After 12 hr. in a refrigerator, the oil solidified, was filtered off and crystallized from isopropyl ether to give 31 g. of O-benzylvanillin (85%), m. p. 63-64° (lit. (41) 63-64°).

3-Methoxy-4-benzyloxynitrostyrene (XCIV). O-Benzylvanillin (30 g.; 0.12 mole), 8.7 g. of ammonium acetate and 45 ml. of nitromethane were dissolved in 90 ml. of glacial

acetic acid. The mixture was refluxed in an oil bath for 1.5 hr. and, after cooling, yellowish needles appeared. The crystals were collected by filtration and recrystallized from 95% ethanol to give 27 g. of 3-methoxy-4-benzyloxynitrostyrene (75%), m.p. 122-123° (lit. (41) 122-123°).

3-Methoxy-4-benzyloxy-phenethylamine hydrochloride (XCV). A solution of 7 g. of 3-methoxy-4-benzyloxynitrostyrene (0.022 mole) in 50 ml. of anhydrous tetrahydrofuran was added over a period of 1 hr. to a stirred solution of 4 g. of lithium aluminum hydride in 150 ml. of ether. Nitrogen was bubbled through the solution during the reaction period. Stirring was continuing for 14 hr. The solution was treated with an excess of a saturated aqueous solution of sodium potassium tartrate. The organic layer was decanted and the aqueous layer extracted twice with 50 ml. of ether. The organic extracts were combined and evaporated to dryness leaving a yellow residue which was dissolved in 50 ml. of anhydrous ether. Dry hydrogen chloride was bubbled into the solution for 2 min. A precipitate was formed, collected and crystallized from 95% ethanol to yield 6.2 g. of the 3-methoxy-4-benzyloxy-phenethylamine hydrochloride (90%), m.p. 171-173° (lit. (41) 173-175°).

N-(3-Methoxy-4-benzyloxyphenethyl)-3-benzyloxy-4-methoxy-phenylacetamide (XCVI). 3-Benzyloxy-4-methoxy-phenylacetic acid (1 g.; 0.0037 mole) was dissolved in 75 ml. of dry benzene, and 10 ml. of thionyl chloride was added. The

mixture was heated at 60° for 2 hr. and stirred for an additional 4 hr. at room temperature. The solvent and the excess of reagent were removed by means of a rotary vacuum evaporator. The residue was dissolved in 30 ml. of anhydrous ether and the solution added dropwise to a solution of 1.1 g. of 3-methoxy-4-benzyloxyphenethylamine hydrochloride in 10 ml. of 10% potassium hydroxide and 15 ml. of ether over a period of 0.5 hr. The stirring was continued for 1 hr. The precipitate was collected and washed with dilute hydrochloric acid (10%) and then with water. It was crystallized from 95% ethanol to give 1.2 g. of a crystalline amide (69.3%), m.p. 136-137° (lit. (41) 134.5-136.5°).

The infrared spectrum was characterized by bands at 3440 cm^{-1} (N-H stretching vibration), 1695 cm^{-1} (carbonyl stretching vibration) and other bands at 1550, 1260 and 1145 cm^{-1}.

1-(3-Benzyloxy-4-methoxybenzyl)-3,4-dihydro-6-methoxy-7-benzyloxyisoquinoline hydrochloride (XCVII). N-(3-Methoxy-4-benzyloxyphenethyl)-3-benzyloxy-4-methoxy-phenylacetamide (1 g.; 0.0019 mole) was dissolved in 12 ml. of dry toluene, and 1 ml. of freshly distilled phosphorus oxychloride was added. The solution was heated in an oil bath at 95-105° in a current of nitrogen, and the solution was evaporated in a rotary vacuum evaporator. Dry benzene was added and evaporated to dryness. The semi-solid residue was dissolved in about 4 ml. of warm 95% ethanol, and 15 ml. of 0.5% hydrochloric acid was added. A precipitation was formed, collected and crystallized from a mixture of chloroform and e-

ther to give 0.81 g. of yellow crystals (81%), m.p. 202-203° (lit. (41) 201-203°).

The infrared spectrum was characterized by bands at 3400 cm^{-1} (=N-H stretching vibration), 1650 cm^{-1} (C=N stretching vibration).

1-(3-Benzyloxy-4-methoxybenzyl)-3,4-dihydro-6-methoxy-7-benzyloxyisoquinoline-methiodide-(N-$^{14}CH_3$) (XCVIII).

The imine hydrochloride (490 mg.; 0.92 millimole) obtained in the previous step was suspended in 25 ml. of ethyl acetate, and 25 ml. of saturated sodium bicarbonate solution was added. Nitrogen was bubbled through the solution until the solid was dissolved. The aqueous layer was extracted with ethyl acetate, and the combined ethyl acetate extracts were washed with water and evaporated to dryness. The residue was dissolved in 6 ml. of dry benzene and transferred to a reaction vessel, 1 cm. in diameter and 10 cm. long. It was connected to a vacuum manifold system through which radioactive iodomethane (2 millicurie/141 mg.) was distilled into the benzene solution. The reaction vessel was sealed off and left at room temperature with occassional shaking. Yellow crystals appeared. After 4 days, the vessel was frozen in liquid nitrogen, and the seal was broken in a dry chamber. An excess of non-radioactive iodomethane was added, and the vessel was sealed again. After 2 days, the crystals were collected. The mother liquid was evaporated to dryness and was worked up separately.

Reticuline-(N-$^{14}CH_3$) (C). The crystals of 1-(3-benzyloxy-4-methoxybenzyl)-3,4-dihydro-6-methoxy-7-benzyloxy-isoquinoline-methiodide-(N-$^{14}CH_3$) were suspended in 20 ml. of methanol, the suspension cooled in an ice bath, stirred with a magnetic stirer, and 600 mg. of sodium borohydride was added in small portions. The reaction mixture was stirred for another 20 min. at room temperature. It was evaporated to dryness, and 20 ml. of water and 1 ml. of 2 N sodium hydroxide were added. The mixture was extracted with ether (4 x 40 ml.) and the combined ether extracts were evaporated to dryness to give a residue of O,O-dibenzylreticuline-(N-$^{14}CH_3$), which was debenzylated without further purification.

The O,O-dibenzyl-reticuline residue (345 mg.) was dissolved in 15 ml. of methanol and 15 ml. of concentrated hydrochloric acid. The solution was heated in an oil bath at 130° for 1 hr., cooled and concentrated to half the volume. It was extracted with ether and the ether extract washed with water.

The combined aqueous layer was basified with concentrated ammonium hydroxide solution to pH 8-9 and extracted with ether. The combined ether extracts were concentrated to dryness to give a residue of 234 mg. This residue was dissolved in ether and a minimum amount of petroleum ether (b.p. 30-60°) was added to cause precipitation. This was repeated several times. The precipitate was collected. Thin layer chromatography of the precipitate on silica gel with chloroform-methanol (9 : 1) and with ethanol-benzene (2 : 8) showed a

single compound identical with natural reticuline isolated from opium. Gas chromatographic analyses of the free base and of the trimethylsilyl derivative on columns containing OV-1 and OV-225 as stationary phases showed the same retention times as natural reticuline. The reticuline so obtained had a specific activity of 3.94 μCi./mg. or 1288.4 millicurie/mole.

2. Synthesis of Laudanine-(N-$^{14}CH_3$) (68)
(see Scheme XII)

3-Benzoyloxy-4-methoxyphenylacetic acid (Benzoylisohomovanillic acid) (CXXXIX). 3-Hydroxy-4-methoxyphenylacetic acid (XC), was dissolved in 10 ml. of 6% sodium hydroxide solution, 0.7 ml. of benzoyl chloride was added, and the mixture was shaken until the odor disappeared. The solution was cooled and acidified with 2 N hydrochloric acid. The oil which appeared, was separated from the aqueous layer and crystallized after standing overnight. The crystals were washed with 96% ethanol to give 1 g. of benzoylisohomovanillic acid, m.p. 126-128° (lit. (68) 128°).

3-Benzoyloxy-4-methoxyphenylacetyl chloride (Benzoylisohomovanillyl chloride) (CXLI). Benzoylisohomovanillic acid (1 g.; 0.0035 mole) was heated in an oil bath at 100° with 5 ml. of thionyl chloride for 1 hr. The excess of reagent was removed on a rotary vacuum evaporator, and the oily residue (1 g.) was dried overnight in a desiccator. It

was used without further purification for the preparation of the amide.

N-(3,4-Dimethoxyphenethyl)-3-benzoyloxy-4-methoxyphenylacetamide (CXLIII). Benzoylisohomovanillyl chloride (1 g.; 0.0033 mole) was dissolved in 4 ml. of distilled chloroform. The solution was cooled to below 5° and slowly added to a cooled solution of 0.6 g. of 3,4-dimethoxyphenethylamine in 10 ml. of chloroform. After 10 min. at 5°, 2.5 ml. of 1 N sodium hydroxide solution was added. The mixture was swirled and the aqueous layer removed with a pipet. The chloroform solution was washed with 2 N hydrochloric acid and then with water, dried over sodium sulfate and evaporated to dryness. Addition of water to the oily residue gave crystals, 1 g. which were filtered off. The product was used without further purification.

1-(3-Benzoyloxy-4-methoxybenzyl)-3,4-dihydro-6,7-dimethoxy-isoquinoline hydrochloride (CXLIV). N-(3,4-dimethoxyphenethyl)-3-benzoyloxy-4-methoxyphenyl-acetamide (1 g.; 0.0022 mole) was dissolved in 50 ml. of dry toluene and 1.5 ml. of phosphorus oxychloride was added. The solution was stirred and maintained at 96-103° for 1¼ hr. and then evaporated to dryness. The residue was dissolved in 8 ml. of absolute ethanol, and dry hydrogen chloride gas was passed through the solution for 2 min. Ether was added, and the precipitate which appeared was collected by filtration to afford 0.7 g. of the imine hydrochloride, m.p. 231-232° (lit.

(68) 232°).

Laudanine-(N-$^{14}CH_3$) (CXLVIII). 1-(3-Benzoyloxy-4-methoxybenzyl)-3,4-dihydro-6,7-dimethoxy-isoquinoline hydrochloride (467 mg.; 1 millimole) was suspended in water, nitrogen was bubbled through the suspension, and sodium bicarbonate was added to a pH of about 8-9. The mixture was extracted repeatedly with ether (4 x 20 ml.), and the combined ether extracts were dried over anhydrous sodium sulfate and evaporated to dryness. The dried residue was dissolved in 5 ml. of benzene and transferred to a methylation vessel, 1 cm. in diameter and 10 cm. long. It was connected to a vacuum manifold system through which radioactive iodomethane (2 millicurie; 141 mg.) was distilled into the benzene solution. The reaction vessel was sealed off and left at room temperature overnight. An oily film appeared on the wall of the tube. After four days the solution was frozen in liquid nitrogen and the seal was broken. An excess of non-radioactive iodomethane (4 ml.) was added. After 24 hr. the crystals were filtered off, suspended in 30 ml. of absolute methanol, and 1 g. of sodium borohydride was added in small portions. The yellow suspension turned colorless. The stirring was continued for 12 hr. The solution was acidified with 5 ml. of 2 N hydrochloric acid, extracted with ether and washed with water. The combined aqueous layer was basified with ammonium hydroxide solution to pH 8-9. It was extracted with ether, the combined ether extract was dried over anhydrous sodium carbonate and concentrated to dryness to give a residue, which was crystal-

lized from a mixture of ethanol and chloroform. Thin layer chromatography of the crystalline compound on silica gel with chloroform-methanol (9 : 1) and with ethanol-benzene (2 : 8) showed it to be identical with natural laudanine isolated from opium. Gas chromatographic analysis of the free base and the trimethylsilyl derivative showed the same retension times as natural laudanine. It had a specific activity of 4.43 μCi./mg. or 1528.4 mCi./mole.

B. Synthesis of Alkaloids for Reverse Isotope Dilution

1. Synthesis of (±)-Coreximine (52)

1,2,3,4-Tetrahydro-7-hydroxy-1-(3-hydroxy-4-methoxybenzyl)-6-methoxyisoquinoline hydrochloride, (±)-Norreticuline hydrochloride (CXXIV). 1-(3-Benzyloxy-4-methoxybenzyl)-3,4-dihydro-6-methoxy-7-benzyloxyisoquinoline hydrochloride (XCVII) (1.1 g.) prepared as described previously (p. 87), was dissolved in 70 ml. of ethanol. The solution was shaken with hydrogen and 500 mg. of palladium (10%) on charcoal at room temperature and at 1 atomosphere's pressure. The uptake of hydrogen was complete in 3 hr. Removal of the catalyst and evaporation of the solution left a residue which solidified on addition of water to give (±)-norreticuline hydrochloride monohydrate, 0.715 g. (94.2%), m.p. 165-166° (lit. (41) 165-166°). The infrared spectrum showed bands at 3250, 1500, 1450, 1300, 1270, 1240, 1120, 1030 and 860 cm^{-1}.

3,10-Dimethoxy-5,6,13,13a-tetrahydro-8-H-dibenzo (a, g) quinolizine-2,11-diol, ((±)-Coreximine) (CIV). (±)-Norreticuline hydrochloride monohydrate (640 mg.; 0.0017 mole) was dissolved in 4 ml. of ethanol. The solution was basified with concentrated ammonium hydroxide solution, and 100 ml. of water was added. It was then extracted with 3 x 40 ml. of chloroform. The combined chloroform extracts were evaporated to dryness to give a residue of 501 mg. The residue was dissolved in 24 ml. of methanol and 5.5 ml. of 37% formaldehyde

solution was added. The solution was refluxed for 1 hr. and the solvent evaporated, leaving a residue which consisted of a mixture of (±)-scoulerine and (±)-coreximine, as evidenced by thin layer chromatography on aluminum oxide with chloroform-methanol (99 : 1) as the solvent. Authentic alkaloids were used for comparison of R_f values.

The residue was dissolved in a minimum amount of chloroform, transferred to a column of neutral alumina (Woelm, activity IV) and eluted with chloroform. The eluate was collected in 10-ml. fractions. Each fraction was analyzed by thin layer chromatography on alumina plates and were compared with authentic samples. Fractions 2 to 5 which contained scoulerine, were combined and evaporated to dryness, to give a residue of 143 mg. Fractions 6 to 12 contained coreximine; these were combined and evaporated to dryness. The residue (300 mg.) was crystallized from methanol, m.p. 237-240° (lit. (52) 238-239°).

The infrared spectrum showed it is identical with natural coreximine.

2. Synthesis of 13-Oxycryptopine (74)

13-Oxycryptopine (CLVI). Cryptopine (CL) oxalate (500 mg.; 1.2 millimole) was dissolved in 100 ml. of warm 0.5 N hydrochloric acid, and concentrated ammonium hydroxide was added to pH 10-11. The solution was extracted with chloroform (5 x 50 ml.) and the combined extracts evaporated to dryness.

The residue (450 mg.) was dissolved in 8 ml. of 2.5% acetic acid, heated to 75°, and 10 ml. of mercuric acetate solution (2.2 g. in 10 ml. of 2.5% acetic acid) was added over a period of 30 min. After 30 min. at 75°, the solution was cooled and filtered to removed mercurous acetate. The filtrate was heated again for 30 min., cooled and filtered, and the process was repeated a third time. The filtrate was basified with concentrated ammonium hydroxide to pH about 10 and extracted with chloroform (3 x 50 ml.). The extract was evaporated to dryness. The residue was purified by preparative thin layer chromatography on silica gel, 2 mm. thickness, with a mixture of chloroform and methanol (17 : 3). The band of oxycryptopine was scraped off, extracted and evaporated to dryness to give a residue. It was crystallized from acetone-petroleum ether (b.p. 30-60°) to give 220 mg. of 13-oxycryptopine, m.p. 187-188° (lit. (74) 186-187°).

The NMR spectrum showed four aromatic protons in the region of 6.6 to 7.6 ppm., two protons of a methylenedioxy group at 6 ppm., six protons of two methoxyl groups at 3.86 to 3.9 ppm., three protons of N-methyl group at 1.86 ppm. (The high field absorption of these N-methyl protons are characteristic of protopine alkaloids.) Signals at 2.2, 2.72 and 3.72 ppm. represent the six methylene protons.

3. Synthesis of (±)-Canadine

(±)-Canadine (CXXIII). Berberine sulfate (500 mg.)

was dissolved in 10 ml. of methanol, 500 mg. of sodium borohydride was added gradually, and the mixture was refluxed for 10 min. After addition of 50 ml. of water, the solution was extracted with chloroform (3 x 30 ml.). The solution was concentrated to dryness to give a residue of 400 mg. It was crystallized from 95% ethanol to give crystalline (±)-canadine, m.p. 136-137° (lit. (83) 134°).

C. Isolation and Purification

1. Extraction of Plants after Administration of (±)-Reticuline-(N-$^{14}CH_3$) and Reverse Isotope Dilution with (±)-Isoboldine, Isocorydine, Tetrahydropalmatine and (+)-Magnoflorine

(a). Preparation of the feeding solution and its administration to Papaver somniferum L. (±)-Reticuline-(N-$^{14}CH_3$) was dissolved in one equivalent of 0.1 N sulfuric acid and diluted with water to give a concentration of about 3 mg. per ml. The solution was injected into the center of the unripe poppy capsule as soon as the petals had fallen, as described by Battersby and Harper (42), each capsule receiving 0.3 ml. corresponding to about 1 mg. of reticuline. Fourteen plants (Indra variety) were fed between June 14 to June 30, 1968, injection being made in 1 to 3 capsules per plant, for a total of 36.6 mg. (144.2 μCi.) of radioactive reticuline. The plants were harvested 8 to 14 days after feeding and placed in a deep-freeze until they could be extracted.

(b). Extraction and isolation of crude alkaloid fractions. The frozen poppy plants were cut into small pieces by means of stainless steel pruning shears and ground to a mash with methanol in a Waring Blender. The slurry was transferred to a glass percolator and (±)-isoboldine (300 mg.), isocorydine hydrochloride (250 mg.), tetrahydropalmatine (250 mg.) and (+)-magnoflorine iodide (400 mg.) were added.

The plant material was macerated for 2 hr. and then percolated with methanol at a speed of 3 to 4 drops per second. This process was continued until the extract was almost colorless and 10 ml. of the percolate —— after concentration —— gave negative test for alkaloids. This required about 10 gallons. The extract was concentrated in a rotary vacuum evaporator to about 1500 ml. and extracted with ethyl acetate (3 x 200 ml.). The combined ethyl acetate extracts were washed with 0.5 N hydrochloric acid (5 x 70 ml.) followed by water (20 ml.). The original aqueous phase and the acid washings were combined and shaken with chloroform (5 x 200 cc.). The combined chloroform extracts were evaporated to dryness to give a residue of 4.88 g. (fraction I).

After addition of concentrated ammonium hydroxide solution to pH 8-9, the aqueous solution was extracted with a mixture of chloroform and isopropyl alcohol (3 : 1) (8 x 200 ml.) and the organic extracts back-washed with water (50 ml.). The aqueous solutions were combined to give fraction II (1500 ml.).

The organic extract was concentrated to dryness and the residue dissolved in 150 ml. of 0.5 N hydrochloric acid. Sodium hydroxide solution (10%) was added to pH about 14 and the solution extracted with chloroform (5 x 200 ml.). The combined extracts were washed with 50 ml. of water and evaporated to dryness to give a residue of 2.2 g. (fraction III).

The pH of the aqueous layer was adjusted to 8 with ammonium chloride and extracted with ether (4 x 200 cc.). The combined ether extracts were dried over anhydrous sodium sulfate

and evaporated to dryness leaving a residue of 1.5 g. (fraction IV).

The aqueous phase, after extraction with ether, was extracted with a mixture of chloroform and isopropyl alcohol (3 : 1) (5 x 300 ml.). The extracts were dried and evaporated to dryness to give a residue of 1.8 g. (fraction V).

(c). Separation and purification.

(1). Fraction I. The residue in fraction I (2.44 g.) was dissolved in 20 ml. of chloroform and transferred to a column of 60 g. silica gel, Woelm, activity 1. The eluate was collected in 150-ml. volumes in Erlenmeyer flasks. The following solvent systems were used.

solvent systems	eluate fractions
benzene	1 - 12
chloroform-ether (1 : 1)	13 - 30
chloroform-methanol (97 : 3)	31 - 80

The eluate fractions were analyzed by thin layer chromatography. Fractions 31 to 46, which were found to contain tetrahydropalmatine, were combined and evaporated to dryness to give a residue labeled Ia (230 mg.).

Fractions 47 to 70, containing isocorydine, gave residue Ib (300 mg.).

Fraction 71 to 80, containing isoboldine, were combined and gave residue Ic (20 mg.).

Residue Ia was crystallized from methanol. Three crystallization were needed to give a constant radioactivity of 11 disintegrations/min./mg. or 1.77 μCi./mole.

The first crystallization gave a specific activity of 13 disintegrations/min./mg., the second,11 disintegrations/min./mg., the third, 11 disintegrations/min./mg.

Residue Ib, the isocorydine residue, was further purified by column chromatography on 50 g. of aluminum oxide, Woelm, activity IV with a mixture of chloroform and benzene (1 : 1). The eluate was collected in 60-ml. volumes in Erlenmeyer flasks. Fractions 2 to 11, containing isocorydine, were combined and evaporated to dryness. The residue was crystallized from a mixture of methanol and water to a specific radioactivity of 6 disintegrations/min./mg. or 0.93 μCi./mole.

The first crystallization gave a specific activity of 374 disintegrations/min./mg., the second, 6 disintegrations/min./mg.

Residue Ic was not further worked up.

(2). Fraction II. Fraction II (1500 ml.) was concentrated under reduced presure to about 200 ml. Half of the volume was acidified with concentrated hydrochloric acid and a saturated solution of ammonium reineckate in water was added slowly to precipitate magnoflorine as a reineckate salt. The precipitate was collected, washed with water and dried in a desiccator. It was then dissolved in 200 ml. of acetone, filtered and a saturated aqueous solution of silver sulfate was added dropwise to the filtrate until no further precip-

itation occurred. The mixture was filtered and the filtrate concentrated to about 20 ml. The precipitate which formed on concentration was removed by filtration and a saturated aqueous solution of potassium iodide was added to the clear filtrate. The solution was refrigerated overnight and the crystals which formed were recrystallized from methanol to constant radioactivity, m.p. 224-225° (lit. (38) 224-225°). Thin layer chromatography on silica gel with a mixture of n-propanol, ammonium hydroxide and water (4 : 1 : 1) gave the same R_f value as an authentic sample of magnoflorine iodide. Three crystallizations were needed to give a radioactivity of 9 disintegrations/min./mg. or 1.92 μCi./mole. The first crystallization gave a specific activity of 49 disintegrations/min./mg., the second, 12 disintegrations/min./mg., the third, 9 disintegrations/min./mg.

Since the radioactivity of magnoflorine was negligible, the other half of the fraction II was not further worked up.

(3). Fraction III. The residue in fraction III was dissolved in 20 ml. of chloroform and transferred to a chromatographic column containing 65 g. of silica gel, Woelm, activity I. The eluate was collected in 200 ml. volumes in Erlenmeyer flasks. The following solvent systems were used.

solvent systems	eluate fractions
chloroform	1 - 28
chloroform-methanol (97 : 3)	29 - 51

Each eluate fraction was analyzed by thin layer chromatography and compared with authentic samples.

Eluate fractions 11 to 18, which contained tetrahydropalmatine, were combined and evaporated to dryness to give residue IIIa (200 mg.).

Eluate fractions 19 to 29 contained isocorydine and gave a residue designated IIIb (50 mg.).

Eluate fractions 30 to 51 contained isoboldine. Combination of fractions 30 to 34, fractions 35 to 45 and fractions 46 to 51, separately, gave residues designated $IIIc_1$ (50 mg.), $IIIc_2$ (100 mg.), $IIIc_3$ (45 mg.). Thin layer chromatography indicated that residue $IIIc_2$ was more pure than the other two. It was crystallized from methanol to give white crystals, 35 mg., having the same R_f value by thin layer chromatography on silica gel as natural isoboldine, m.p. 122-123° (lit. (20) 122-123°)

Four crystallizations were needed to give a constant radioactivity of 900 disintegrations/min./mg. or 132 μCi./mole. The first crystallization gave a specific activity of 975 disintegrations/min./mg., the second, 904 disintegrations/min./mg., the third, 895 disintegrations/min./mg., the fourth, 900 disintegrations/min./mg.

(4). Fraction IV. Fraction IV was subjected to column chromatography on silica gel. Impure isoboldine was obtained. No further purification was carried out.

(5). Fraction V. Fraction V which consisted mainly of morphine, was not worked up.

2. Extraction of Plants after Administration of (±)-Reticuline-(3-^{14}C) and Reverse Isotope Dilution with (±)-Canadine, (±)-Coreximine and (±)-Isoboldine

(a). Preparation of the feeding solution and its administrations to Papaver sommiferum L. The solution of labeled reticuline was prepared and fed to opium poppies (Noordster variety) and the plants harvested as described on page 98. Seventeen plants were fed a total of 19.9 mg. (80.3 μCi.) from October 19 to November 10, 1967.

(b). Extraction and isolation of crude alkaloid fractions. The plants were ground with methanol in a Waring Blender, and (±)-canadine (200 mg.), (±)-coreximine (210 mg.) and (±)-isoboldine (200 mg.) were added and the material extracted as already described (p. 98). The methanol extract (about 10 gallons) was concentrated under reduced pressure to 900 ml. and extracted with ethyl acetate (3 x 200 ml.). The ethyl acetate layer was washed with 1 N hydrochloric acid (4 x 300 ml.). The combined aqueous solutions were extracted with chloroform (10 x 300 ml.). Evaporation of the chloroform gave a residue of 6.5 g. (fraction I).

The aqueous solution was adjusted to pH 8-9 with sodium bicarbonate and extracted with chloroform (5 x 200 ml.). Evaporation of the chloroform gave a residue of 1.2 g. (fraction II).

(c). Separation and purification.

(1). Fraction I. One-half of the residue in fraction I (3.25 g.) was dissolved in 10 ml. of benzene and transferred to a chromatographic column containing of 60 g. silica gel, Woelm, activity I. The eluate was collected in 50-ml. volumes in Erlenmeyer flasks. The following solvent systems were used.

solvent systems	eluate fractions
benzene-methanol (98 : 2)	1 - 11
benzene-methanol (95 : 5)	12 - 23

Each eluate fraction was analyzed by thin layer chromatography and compared with authentic samples.

Eluate fractions 12 to 23, which were found to contain canadine, were combined and evaporated to dryness to give a residue labeled Ia (200 mg.). This residue was further purified by column chromatography on 50 g. of neutral aluminum oxide, Merck, activity III. The eluate was collected in 50-ml. fractions. The following solvent systems were used.

solvent systems	eluate fractions
hexane	1 - 2
benzene	3 - 9
benzene-chloroform	10 - 12

Eluate fractions 4 to 7, containing canadine, were com-

bined and evaporated to dryness to give a residue weighing 40 mg. Crystallization from 95% ethanol gave pale yellowish granular crystals. Constant radioactivity of 10 disintegrations/min./mg. or 1.54 μCi./mole was achieved after six crystallizations. The six crystallizations gave the following specific activity:

no. of crystallization	disintegrations/min./mg.
1	1255
2	241
3	44
4	15
5	12
6	10

(2). Fraction II. Thin layer chromatography showed that fraction II contained isoboldine and coreximine. These alkaloids were separated by preparative thin layer chromatography on silica gel (20 cm. x 20 x 2 mm.). About 500 mg. of the residue was applied to each plate and the chromatograms developed with a mixture of chloroform and methanol (9 : 1). The air in the glass jar was replaced with nitrogen before the solvent was added and the chromatograms were protected from light during development. An iodine spray along the margin was used for detection of the alkaloid bands. The bands corresponding to coreximine and isoboldine were collected separately and the silica gel was extracted with warm methanol. Evaporation of the solvent gave residues IIa_1 (270 mg.) and IIb (210 mg.). Thin layer chromatography showed that IIa_1 consisted mainly of isoboldine with a small amount

of coreximine and some impurities, whereas IIb contained mostly coreximine with a small amount of isoboldine and some impurities.

The IIa_1 residue was further purified by preparative thin layer chromatography on silica gel (20 cm. x 20 x 2 mm.). About 130-140 mg. of residue was applied to each plate and the chromatograms were developed with a mixture of benzene and ethanol (8 : 2). The band corresponding to isoboldine was scraped off and the alkaloid was extracted with warm methanol. Evaporation of the solvent gave residue IIa_2 (40 mg.). Attempts to crystallize residue IIa_2 were unsuccessful.

Residue IIa_2 (15 mg.) was dissolved in 5 ml. of ether and an excess of iodomethane was added. The solution was left in a refrigerator overnight. A precipitate was formed, which could not be induced to crystallize.

Residue IIa_2 (25 mg.) was treated with an excess of diazomethane in ether to give glaucine. The mixture was left at room temperature for two days. It was then evaporated to dryness and the residue purified by preparative thin layer chromatography on silica gel with a mixture of chloroform and methanol (9 : 1). The purified glaucine was dissolved in 20 ml. of ether and an excess of iodomethane was added. When the mixture was left overnight, crystals of glaucine methiodide appeared and were crystallized four times from methanol-petroleum ether (b.p. 30-60°) to a constant radioactivity of 429 disintegrations/min./mg. or 94.2 μCi./mole. The four crystallization gave the following activity:

no. of crystallization	disintegrations/min./mg.
1	687
2	552
3	434
4	429

Residue IIb which contained coreximine as the major constituent, was dissolved in a minimum anount of chloroform, transferred to a chromatographic column containing 30 g. of aluminum oxide, Merck, activity IV and eluted with chloroform. The eluate was collected in 25 ml. volumes in test tubes. Each tube was checked by thin layer chromatography by comparison with authentic coreximine. Tubes 8 to 15, which contained coreximine were combined, evaporated to dryness and the residue crystallized seven times from methanol to constant radioactivity of 1480 disintegrations/min./mg. or 220 μCi./mole, m.p. 237-240° (lit. (52) 238-239°). Thin layer chromatography on aluminum oxide showed it to be identical with an authentic sample. The seven crystallizations gave the following radioactivity:

no. of crystallizations	disintegrations/min./mg.
1	2318
2	1575
3	1692
4	1745
5	1514
6	1478
7	1480

3. Extraction of Plants after Administration of (±)-Laudanine-(N-$^{14}CH_3$) and Reverse Isotope Dilution with (±)-Laudanosine

(a). Preparation of the feeding solution and its administration to Papaver somniferum L. The solution of (±)-laudanine-(N-$^{14}CH_3$) was prepared and fed to opium poppies (Indra variety) and the plants harvested as described for reticuline. Seven plants were fed a total of 10 mg. (43.3 µCi.) in August 1968.

(b). Extraction and isolation of the crude alkaloid fractions. The plants were ground in a Waring Blender with methanol, 200 mg. of (±)-laudanosine was added as isotope diluent and the material extracted as described previously (p. 98). The methanol extract (about 4 gallons) was concentrated to 200 ml. under reduced pressure and extracted with ethyl acetate (2 x 100 ml.). The ethyl acetate layer was back-extracted with 0.1 N hydrochloric acid (3 x 100 ml.). The combined acid and aqueous layers were made alkaline with 10% sodium hydroxide solution to pH 14 and extracted with ether (8 x 100 ml.). The ether was removed by evaporation to give a residue of 600 mg.

(c). Separation and purification. Since the plants had been fed radioactive laudanine, it was necessary to use a purification method which would effectively remove traces of laudanine from the isolated laudanosine. Several solvent

systems were studied by thin layer chromatography on alumina. A mixture of chloroform and isopropyl ether (1 : 1) gave good separation of the two alkaloids and was, therefore, chosen for purification of the extract by column chromatography.

The residue (600 mg.) was dissolved in a minimum amount of chloroform, transferred to a chromatographic column containing 20 g. of aluminum oxide, Merck, activity IV and eluted with a mixture of chloroform and isopropyl ether (1 : 1). The eluate was collected in 15-ml. volumes in test tubes. Each tube was checked by thin layer chromatography and compared with authentic alkaloid samples. Tubes 3 to 9, which contained laudanosine, were combined and evaporated to dryness to give 100 mg. of residue. It was crystallized seven times from ether to give a constant radioactivity of 768 disintegrations/min./mg. or 123.5 μCi./mole, m.p. 88-89° (lit. (83) 89°). The seven crystallizations gave specific activity as follows:

no. of crystallization	disintegrations/min./mg.
1	773
2	869
3	787
4	889
5	749
6	758
7	768

4. Extraction of Plants after Administration of (±)-Reticuline-(N-$^{14}CH_3$) and Reverse Isotope Dilution with Oxycryptopine, Boldine, Sinomenine Hydrochloride, Berberine Sulfate and Glaucine.

(a). Preparation of feeding solution and its administration to Papaver somniferum L. The solution of (±)-reticuline-(N-$^{14}CH_3$) was prepared and fed to opium poppies (Indra variety) and the plants harvested as described previously (p. 98). Seventeen plants were fed a total of 34.1 mg. (134.35 μCi.) in June of 1969.

(b). Extraction and isolation of crude alkaloid fraction. The plants were ground with methanol in a Waring Blender, and oxycryptopine (250 mg.), boldine (250 mg.) sinomenine hydrochloride (275 mg.), berberine sulfate (250 mg.) and glaucine (250 mg.) were added. The material was extracted and a partial separation of alkaloids performed by liquid-liquid extraction as described on page 98. Each fraction was analyzed for alkaloids by thin layer chromatography. The weight and the main alkaloid contents of each fraction were:

fraction I	(4 g.) glaucine
fraction II	(aqueous) inorganic salts
fraction III	(1.6 g.) sinomenine, oxycryptopine and berberine
fraction IV	(0.7 g.) boldine
fraction V	(1.5 g.) boldine and morphine

(c). Separation and purification.

(1). Fraction I. Fraction I (4 g.) was dissolved in 25 ml. of chloroform and transferred to a chromatographic column containing 85 g. of silica gel, Woelm, activity I. The eluate was collected in 200-ml. volumes in Erlenmeyer flasks. The following solvent systems were used.

solvent systems	eluate fractions
chloroform	1 - 3
chloroform-methanol (99 : 1)	4 - 12
chloroform-methanol (96 : 4)	13 - 40

Each eluate fraction was checked by thin layer chromatography and compared with authentic alkaloids.

Eluate fractions 13 to 27, which were found to contain glaucine, were combined and the solvent removed by evaporation to give an oily residue, designated Ia (450 mg.). Attempts to crystallize it were unsuccessful.

The residue Ia was further purified by preparative thin layer chromatography on silica gel (20 cm. x 20 x 2 mm.) with a mixture of chloroform and methanol (9 : 1). In this way an oily residue of glaucine (250 mg.) was obtained. It would still not crystallize.

The oil was dissolved in 2 ml. of methanol and 10 ml. of iodomethane was added, the mixture was left in room temperature for 24 hr. and then concentrated to 2 ml. Ether was added

dropwise till turbid and granular crystals of glaucine methiodide appeared on cooling. They were recrystallized from methanol-ether, m.p. 224-225° (lit. (83) 224-225°). After four crystallizations it gave a specific activity of 6 disintegrations/min./mg. or 0.97 μCi./mole. The four crystallizations gave the following radioactivity.

no. of crystallization	disintegrations/min./mg.
1	191
2	36
3	14
4	6

(2). Fraction III. Fraction III (1.6 g.) was dissolved in 10 ml. of chloroform and transferred to a chromatographic column containing 65 g. of silica gel, Woelm, activity I. The eluate was collected in 200-ml. volumes in Erlenmeyer flasks. The following solvent systems were used.

solvent systems	eluate fractions
chloroform	1 - 6
chloroform-methanol (98 : 2)	7 - 11
chloroform-methanol (96 : 4)	12 - 57
chloroform-methanol (95 : 5)	58 - 90

Each eluate fraction was checked by thin layer chromatography and compared with authentic alkaloids.

Eluate fractions 13 to 32 were found to contain sino-

menine. The contents were combined and evaporated to dryness to give a residue labeled IIIa (300 mg.). This residue was dissolved in 10 ml. of benzene and about 15 ml. of ether was added, precipitation occurred. The precipitate was collected and crystallized seven times from benzene to a constant radioactivity of 11 disintegrations/min./mg. or 1.64 μCi./mole, m.p. 160-162° (lit. (83) 162°). The seven crystallizations gave the following radioactivity:

no. of crystallization	disintegrations/min./mg.
1	1999
2	936
3	60
4	34
5	28
6	12
7	11

Eluate fractions 33 to 77 contained oxycryptopine. The contents were combined and evaporated to give a residue labeled IIIb (250 mg.).

Eluate fractions 78 to 108 contained berberine. These fractions were concentrated to 100 ml. and 2 ml. of 10% sulfuric acid was added. It was further concentrated to about 2 ml. Yellow crystals appeared. The crystals were collected on a filter and were crystallized twice from 95% ethanol, yielding a specific activity of 7 disintegrations/min./mg. or 1.23 μCi./mole. The first crystallization gave a specific activity of 14 disintegrations/min./mg., the second crystallization gave a specific activity of 6 disintegrations/min./mg.

Residue IIIb was further purified by column chromatography on 20 g. aluminum oxide, Woelm, activity IV. The eluate was collected in 5-ml. volumes in test tubes. The following solvent systems were used.

solvent systems	eluate fractions
benzene-chloroform (1 : 1)	1 - 14
chloroform	15 - 54
chloroform-methanol (9 : 1)	55 -

The eluate fractions 15 to 54 contained oxycryptopine. These fractions were concentrated and evaporated to dryness. The residue was crystallized from acetone-petroleum ether, (b.p. 30-60°), m.p. 187-188° (lit. (83) 186-187°). It was recrystallized six times to give a specific radioactivity of 10 disintegrations/min./mg., or 1.74 μCi./mole. The seven crystallizations gave the following activity:

no. of crystallization	disintegrations/min./mg.
1	1254
2	300
3	85
4	38
5	31
6	15
7	10

(3). Fraction IV. Thin layer chromatography showed that fraction IV contained boldine. It was purified

by preparative thin layer chromatography on silica gel (20 cm. x 20 x 2 mm.). The chromatograms were developed with a mixture of chloroform and methanol and were protected from light during the development. The band corresponding to boldine was collected, and the silica gel was extracted with warm methanol. Evaporation of the solvent gave a residue which was crystallized seven times from methanol to give a specific radioactivity of 20 disintegrations/min./mg., or 2.97 μCi./mole. The seven crystallizations gave the following radioactivity:

no. of crystallization	disintegrations/min./mg.
1	3901
2	735
3	333
4	131
5	46
6	35
7	20

D. Degradation of Radioactive Compounds

1. Degradation of Radioactive Coreximine (see Scheme X)

The degradation of coreximine was carried out both with non-radioactive coreximine and radioactive coreximine (isolated from plants fed with reticuline-(3-^{14}C)).

2,11-Dihydroxy-3,10-dimethoxy-7-methyl-5,6,13,13a-tetrahydro-8-H-dibenzo (a,g) quinolizinium iodide ((±)-phellodendrine iodide) (CXXVIII). Coreximine (55 mg.) was dissolved in 5 ml. of methanol and 3 ml. of iodomethane was added. The mixture was refluxed in a water bath. After 2 hr., the solution was evaporated to dryness. Addition of 1 ml. of warm methanol gave yellow crystals of (±)-phellodendrine iodide, 56 mg., m.p. >300° (lit. (84), m.p. > 300°).

2,11-Diethoxy-3,10-dimethoxy-7-methyl-5,6,13,13a-tetrahydro-8-H-dibenzo (a,g) quinolizinium iodide ((±)-O,O-diethylphellodendrine iodide) (CXXIX). The crystals (±)-phellodendrine iodide were dissolved in 4 ml. of 1 N alcoholic potassium hydroxide solution and 4 ml. of iodoethane was added. The solution was refluxed in a water bath for 2 hr. Another 2 ml. of 1 N alcoholic potassium hydroxide and 2 ml. of iodoethane was added and the reflux continued for 2 hr. The resulting solution was evaporated to dryness to give a yellowish oily residue of O,O-diethylphellodendrine iodide (58 mg.).

O,O-Diethylphellodendrine methine (CXXX). The

residue (58 mg.) of 0,0-diethylphellodendrine iodide was dissolved in 3 ml. of ethanol. Four grams of potassium hydroxide in 10 ml. of water was added. The solution was refluxed in an oil bath at 110-120° for 3 hr. and extracted with chloroform (3 x 20 ml.). The combined chloroform extracts were concentrated to dryness to give a residue of 0,0-diethylphellodendrine methine (34 mg.). Thin layer chromatography of the residue showed a major spot corresponding to 0,0-diethylphellodendrine methine obtained from the inactive run, which had the m.p. of 116-117° (lit. (84) 116-117°).

Ozonolysis. The residue of 0,0-diethylphellodendrine methine (CXXX) was dissolved in 5 ml. of ethyl acetate, cooled in a dry ice-chloroform bath and ozonized oxygen was passed through the solution at a rate of 2 bubbles per second over a period of 15 min. Finally, oxygen was past through the resulting blue solution for 5 min. to expel excess of ozone. The solution became colorless and was evaporated to dryness to give an oily residue. Zinc dust (0.2 g.), 20 ml. of water and 10 mg. of silver nitrate were added. The mixture was refluxed in an oil bath at 115° to 120° for 30 min. Half of the water was then distilled at atmospheric pressure into a solution of 0.15 g. (0.0011 mole) dimedone in 10 ml. of water and 4 ml. of ethanol. Water (10 ml.) was added to the distilling flask and the distillation continued. The formaldehyde-dimedone which separated over a period of 15 hr., was collected to give 16 mg. of crystals. It was recrystal-

lized three times from 50% aqueous ethanol to a constant radioactivity of 1423 disintegrations/min./mg., or 196 μCi./mole. The first crystallization gave 1218 disintegrations/min./mg., the second, 1404 disintegrations/min./mg. and the third, 1423 disintegrations/min./mg.

2. N-Demethylation of Laudanosine

Laudanosine (37.5 mg., isolated from (±)-laudanine-(N-$^{14}CH_3$) fed plants), 50 mg. of ammonium iodide, 500 mg. of phenol (as a solvent), 5 drops of chloroauric acid (prepared as a solution of 0.1 g., in 3.5 ml. of water) and 2 ml. of freshly distilled hydroiodic acid (distilled from red phosphorus) were placed in the flask of a modified Clark alkoxy apparatus. The mixture was allowed to stand at room temperature for 20 min. in a current of nitrogen after which time it was heated gradually to 180° in a bath of Wood's metal. The nitrogen gas, with liberated methyl iodide, passed through a solution consisting of a mixture of 5% sodium thiosulfate and 5% cadmium sulfate (1 : 1) into a trap containing a 10% solution of triethylamine in ethanol (5 ml.). The trap was immersed in a bath of chloroform and dry ice. The temperature of the Wood's metal bath was maintained at 180-195° for 1 hr. during which time O-demethylation occurred. The mixture was then allowed to stand at room temperature for 30 min. The triethylamine solution in the trap was replaced with a new solution, and the temperature of the Wood's

metal bath was raised slowly to 360° where it was maintained for 1 hr. for N-demethylation. The triethylamine solutions from O- and N-demethylation were allowed to stand overnight and were evaporated to dryness.

The yield from O-demethylation was 100 mg. of triethylmethylammonium iodide (102%).

The yield from N-demethylation was 25 mg. of triethylmethylammonium iodide (102%).

Both residues were crystallized from ethanol and ether to constant radioactivity. The triethylmethylammonium iodide from O-demethylation was inactive.

The triethylmethylammonium iodide from N-demethylation had specific activity of 1128 disintegrations/min./mg. or 123.9 μCi./mole. The first crystallization gave specific activity of 1119 disintegrations/min./mg., the second crystallization gave specific activity of 1128 disintegrations/min./mg.

3. N-Demethylation of Isoboldine

Isoboldine (33 mg.) isolated from (±)-reticuline-(N-$^{14}CH_3$) fed plants, was demethylated in a modified Clark apparatus as described for demethylation of laudanosine with the exception that the temperature was kept at 180-195° for 1.5 hr. instead of 1 hr. for complete O-demethylation. The resulting triethylamine solutions of both N- and O-demethylation were kept overnight at room temperature and evaporated to dryness to give residues of triethylmethylammonium iodide.

The yield from O-demethylation was 29 mg. of triethylmethylammonium iodide (100%).

The yield from N-demethylation was 14 mg. of triethylmethylammonium iodide (115%).

The high yield of triethylmethylammonium iodide from the N-demethylation was probably caused by distilled hydroiodic acid reacting with triethylamine to form the triethylammonium iodide. However, it was easily purified by crystallization from a mixture of ethanol and ether. The triethylmethylammonium iodide from O-demethylation was inactive, whereas that from N-demethylation, after four crystallizations, had a specific activity of 1194 disintegrations/min./mg. or 131.2 μCi./mole. The third crystallization gave specific activity of 1185 disintegrations/min./mg. The fourth crystallization gave specific activity of 1194 disintegrations/min./mg.

SUMMARY

The objective of this study was to investigate the biosynthetic pathways and mechanisms of certain opium alkaloids, and to explore the presence of new alkaloids in Papaver somniferum based on a biosynthetic approach.

Radioactive precursors, (±)-reticuline-(N-$^{14}CH_3$), (±)-reticuline-(3-^{14}C), (±)-codamine-(3-^{14}C) and (±)-laudanine-(N-$^{14}CH_3$) were synthesized and fed to opium poppies. Reverse isotope dilution technique was applied to isolate the desired alkaloids. Isoboldine, coreximine, canadine and oxycryptopine were synthesized and used for isotope dilution purposes, the other alkaloids were obtained from commercial sources.

I. Biosynthetic Studies

(A). Barton and Cohen proposed that aporphines are produced in plants from a benzyltetrahydroisoquinoline by a phenol oxidation via an intermediate quinoid biradical. The possible mechanisms were envisaged, either a direct coupling or formation of an intermediate dienone which in turn might rearrange to give rise to a great variety of aporphines.

When (±)-reticuline-(3-^{14}C) was administed to opium poppies, it was incorporated into isoboldine to an extent of 0.073%. The feeding experiment was repeated with (±)-reticuline-(N-$^{14}CH_3$), isoboldine and magnoflorine being used for isotope dilution. Again, isoboldine showed good incorporation (0.084%) of radioactivity whereas magnoflorine was inactive.

Since reticuline, isoboldine and magnoflorine are known to exist in the opium poppy, it may be concluded that aporphines with 1, 2, 9, 10 substitution pattern (isoboldine type) are biosynthesized by a direct phenol coupling, while this is not the case for aporphines with substituents at positions 1, 2, 10 and 11 (corytuberine type). Because of steric factors, these aporphines are more likely to be biosynthesized *via* dienone intermediate, followed by dienone-phenol rearrangement.

(B). By feeding opium poppies with (±)-codamine-(3-^{14}C) and (±)-laudanine-(*N*-$^{14}CH_3$), it was found that the major pathway leading from reticuline to laudanosine is by way of laudanine.

Reticuline ⟶ codamine ⟶ laudanosine represents only a minor pathway.

II. Characterization of New Opium Alkaloids

(A). It has been shown that protoberberines are produced in plants from (+)-reticuline in such a way that the *N*-methyl group of reticuline becomes the C-8 methylene group of the protoberberine. We have shown in this study that the tetrahydro-ψ-berberine, coreximine, is also derived from reticuline. (±)-Reticuline-(3-^{14}C) was incorporated into coreximine to an extent of 0.174%. Controlled degradation showed that the radioactivity was located at the C-6 position, as expected. Consequently, it could be concluded that the opium poppy is capable of converting reticuline to coreximine, and that coreximine, like scoulerine and isocorypalmine, is a normal mem-

ber of the opium alkaloids. In the same way, it was shown that canadine, tetrahydropalmatine and berberine were not present in the plant.

(B). Our tracer studies showed no incorporation of (±)-reticuline-(N-$^{14}CH_3$) into glaucine, isocorydine, boldine, sinomenine and oxycryptopine. It would, therefore, seem logical to conclude that the opium poppy does not contain glaucine, boldine, sinomenine and oxycryptopine. No decision can be made, however, regarding the presence of isocorydine, since it, like corytuberine and magnoflorine, does not appear to be biosynthesized from reticuline in this plant.

BIBLIOGRAPHY

1. G. Sertürner, Ann. Phys., 55, 56 (1817)
2. S. A. E. Hakim, V. Mijovic and J. Walker, Nature, 189, 198 (1961)
3. R. Robinson, "The Structural Relations of Natural Products", Clarendon, Oxford, 1955
4. R. Robinson and S. Sugasawa, J. Chem. Soc. , 3163 (1931)
5. E. Winterstein and G. Trier, "Die Alkaloide", Borntträger, Berlin , 1910
6. A. R. Battersby, "Tilden Lecture", Proc. Chem. Soc. , 189 (1963)
7. D. H. R. Barton, R. H. Hesse and G. W. Kirby, Proc. Chem. Soc., 267 (1963)
8. D. H. R. Barton, R. H. Hesse and G. W. Kirby, J. Chem. Soc., 6379 (1965)
9. D. H. R. Barton, G. W. Kirby, W. Steglich, G. M. Thomas, A. R. Battersby, T. A. Dobson and H. Ramuz, J. Chem. Soc., 2423 (1965)
10. A. R. Battersby and B. J. T. Harper, Chem. and Ind. , 365 (1958)
11. E. Brochmann-Hanssen and T. Furuya, Planta Medica, 12, 328 (1964)
12. D. H. R. Barton and T. Cohen, "Festschrift Arthur Stoll", Birkhauser, Basel, 1957, p. 117
13. R. Robinson, J. Chem. Soc., 111, 876 (1917)
14. M. M. Nijland, Pharm. Weekbl., 99, 1165 (1964)

15. E. Brochmann-Hanssen, B. Nielsen and K. Hirai, J. Pharm. Sci., 56, 754 (1967)

16. H. Avenarius and R. Pschorr, Chem. Ber., 62, 321 (1929)

17. D.H. Hey and L. C. Lobo, J. Chem. Soc., 2246 (1954)

18. B. Franck, G. Blaschke and G. Schlingloff, Tetrahedron Letters, 439 (1962)

19. S. M. Albonico, A. M. Kuck and V. Deulofeu, Chem. and Ind., 1580 (1964)

20. A. H. Jackson and J. A. Martin, Chem. Commun., 420 (1965)

21. T. Kametani, T. Sugahara, H. Yagi and K. Fukumoto, Tetrahedron, 25, 3667 (1969)

22. H. Plieninger and G. Keilich, Chem. Ber., 91, 1891 (1958)

23. K. V. Auwers and K. Ziegler, Ann. Chem., 425, 217 (1921)

24. A. H. Jackson and J. A. Martin, Chem. Commun., 142 (1965)

25. M. Shamma and W. A. Slusarchyk, Chem. Commun., 528 (1965)

26. A. R. Battersby and T. H. Brown, Proc. Chem. Soc., 85 (1964)

27. A. R Battersby, T. H. Brown and J. H. Clements, J. Chem. Soc., 4550 (1965)

28. T. K. Kametani and H. Yagi, J. Chem. Soc., 2182 (1967)

29. M. P. Cava, Chem. Rev., 68, 321 (1968)

30. I. R. C. Bick, J. Harley-Mason, N. Sheppard and M. J. Vernengo, J. Chem. Soc., 1900 (1961)

31. M. Shamma, Experientia, 16, 484 (1960)

32. C. Djerassi, K. Mislow and M. Shamma, Experientia, 18, 53 (1962)

33. A. R. Battersby, R. T. Brown, J. H. Clements and G. G. Iverach, Chem. Commun., 230 (1965)

34. A. R. Battersby and T. H. Brown, Chem. Commun, 170 (1966)

35. D. H. R. Barton, D. S. Bhakuni, G. M. Chapman and G. W. Kirby, J. Chem. Soc., 2134 (1967)

36. L. J. Haynes, D. H. R. Barton, D. S. Bhakuni and G. W. Kirby, Chem. Commun., 141 (1965)

37. G. Blaschke, Arch. Pharm., 432 (1968)

38. M. S. Flom, R. W. Doskotch and J. K. Beal, J. Pharm. Sci., 56, 1515, 1967

39. M. Sribney and S. Kirkwood, Nature, 171, 931 (1953)

40. D. H. R. Barton, G. W. Kirby and J. B. Taylor, J. Chem. Soc., 4545 (1963)

41. A. R. Battersby, R. Binks, R. J. Francis, D. J. McCaldin and H. Ramuz, J. Chem. Soc., 3600 (1964)

42. A. R. Battersby, R. Binks and B. J. T. Harper, J. Chem. Soc., 3534 (1962)

43. H. Boit, "Ergebnisse der Alkaloid-Chemie Bis 1960", Akademie-Verlag, Berlin , 1961, p. 288

44. S. Pfeifer and J. Teige, Pharmazie, 17, 692,(1962)

45. S. Pfeifer, Pharmazie, 21, 492,(1966)

46. E. Brochmann-Hanssen, B. Nielsen and K. Hirai, unpublished paper.

47. E. Brochmann-Hanssen and B. Nielsen, Tetrahedron Letters, 2261 (1966)

48. S. Ose, H. Kaneko and K. Namba, Japan Patent 15364, C.A., 55, 3933 (1961)

49. A. Pictet and T. Q. Chou, Chem. Ber., 49, 370 (1916)

50. E. Späth and E. Kruta, Chem. Ber., 62, 1024 (1929)

51. A. R. Battersby, R. Southgate, J. Staunton and M. Hirst,

J. Chem. Soc., 1052 (1966)

52. T. Kametani and K. Fukumoto, J. Chem. Soc., 112 (1968)

53. J. R. Gear and I. D. Spenser, Can. J. Chem., 41, 783 (1963)

54. D. H. R. Barton, R. H. Hesse and G. W. Kirby, Proc. Chem. Soc., 267 (1963)

55. A. R. Battersby, R. J. Francis, M. Hirst and J. Staunton, Proc. Chem. Soc., 268 (1963)

56. A. R. Battersby, Proc. Chem. Soc., 189 (1963)

57. D. H. R. Barton, Proc. Chem. Soc., 293 (1963)

58. D. H. R. Barton, R. H. Hesse and G. W. Kirby, J. Chem. Soc., 6379 (1965)

59. P. W. Jeffs, in Manske and Holmes (editors) "The Alkaloids" Vol. IX 1967 Academic Press, New York, p. 96

60. A. R. Battersby, R. J.Francis, M. Hirst and R. Southgate, Chem. Commun., 89 (1965)

61. R. H. F. Manske, Can. J. Research, 16B, 81 (1938)

62. A. R. Battersby, P. Böhler, M. H. G. Munro and R. Ramage, Chem. Commun., 1066 (1969)

63. O. Hesse, Ann. Chem., 153, 47 (1870)

64. O. Hesse, Ann. Chem., 282, 208 (1894)

65. E. Brochmann-Hanssen and B. Nielsen, Tetrahedron Letters, 1271(1965)

66. H. Corrodi and E. Hardegger, Helv. Chim. Acta., 39, 889 (1956)

67. A. R. Battersby, D. M. Foulkes and R. Binks, J. Chem. Soc., 3323 (1965)

68. V. Deulofeu, Tetrahedron, 4, 342 (1958)

69. J. Smith, J. Pharm., 8, 595 (1867)

70. O. Hesse, Chem. Ber., 4, 693 (1871)

71. E. Brochmann-Hanssen, B. Nielsen, J. Pharm. Sci., 55, 743 (1966)

72. R. H. F. Manske, in Manske and Holmes (editors) "The Alkaloids", Vol. IV, 1954, Academic Press, New York, p. 5

73. R. H. F. Manske and L. Marion, J. Am. Chem. Soc., 62, 2042 (1940)

74. E. Brochmann-Hanssen and A. Leung, Planta Medica, in press

75. M. Maturová, Vl. Preininger and F. Santavý, Planta Medica, 16, 121 (1968)

76. E. Brochmann-Hanssen, A. Leung and W. J. Richter, Planta Medica, in press

77. D. H. R. Barton, D. S. Bhakuni, R. James and G. W. Kirby, J. Chem. Soc., 128 (1967)

78. H. Rapoport, F. R. Stermitz and D. R. Baker, J. Am. Chem. Soc., 82, 2765 (1960)

79. F. R. Stermitz and H. Rapoport, Nature, 189, 310 (1961)

80. A. R. Battersby and B. J. T. Harper, Tetrahedron Letters, 21 (1960)

81. A. R. Battersby, G. W. Evans, R. O. Martin, M. E. Warren Jr. and H. Rapoport, Tetrahedron Letters, 1275 (1965)

82. D. H. R. Barton, A. J. Kirby and G. W. Kirby, J. Chem. Soc., 929 (1968)

83. Dictionary of Organic Compounds, 4th edition, Oxford University Press, New York, 1965

84. M. Tomita and J. Kunitomo, J. Pharm. Soc. Japan, 80, 1238, (1960)